# PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION ON THE LEAVES OF *Ipomoea pes-tigridis* Linn. (CONVOLVULACEAE)

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**Dr. (Mrs). AJITHADAS ARUNA, M. Pharm., Ph. D.,**
**PRINCIPAL,**
**College of Pharmacy,**
**Madurai Medical College,**
**Madurai-625020**

## CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *Ipomoea pes-tigridis* Linn. (CONVOLVULACEAE)''** submitted by **Mrs. S. SAMEEMA BEGUM (Reg. No. 26108667)** in partial fulfilment of the requirement for the award of the degree of **MASTER OF PHARMACY** in **PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R. Medical University is a bonafied work done by her under my guidance during the academic year 2011-2012 at the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai-625020.

Station : Maduari (**Mrs. AJITHADAS ARUNA)**

Date :

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# CHAPTER-1

## INTRODUCTION

India has an immense wealth of rich biodiversity knowledge about ethno medicine which has been accumulated by villagers and tribal people that are unknown to "scientist and urban people". Man has been using herbs and plant products for combating diseases from ancient times.

Drugs used in medicine today are either obtained from nature or of synthetic origin. In the last 3 decades significant events occurred that have already produced fundamental changes in the attitude of both the public and scientists towards herbal medicine. Dissatisfaction with the effectiveness and the cost of modern medicine caused an appreciation for all things natural to millions of people throughout the world for the use of plant drugs for the treatment of various ailments [1].

### Tribal Medicine

The mother of all indigenous medicine is tribal medicine. The study under taken during 1994-1998 with the support of IDRC (International Development Research Centre, New Delhi) has discovered that there are about 45,000 species of wild plants. Out of this, 7,500 species are in medicinal use in health practice of tribals who are in harmony with nature.

Numerous types of herbs have been well recognised and catalogued by botanists from the high range of Himalayan tract up to the seashore of Kanyakumari. This extensive flora has been greatly utilised as a source of drugs in the Indian Traditional Medicine [2].

### Traditional medicine

In India, the use of medicinal plants were found to be in the Rigveda which was written between 4500-1600BC. Initially all drugs were natural, such as vegetable, animal and mineral products in their crude forms. Before the emergence of the twentieth century, all

medical practice was what we now call the traditional system. The World Health Organization (WHO) has defined traditional medicine as "the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing" (WHO, 1978). Traditional medicine is therefore, used mainly to distinguish the ancient and culture-bound health care practices, which existed before the application of science to health matters in official modern scientific medicine or allopathy. The 75% Indian population depend on this indigenous system of medicine. With a huge section of ever increasing population relying on herbal remedies, it is imperative that plant products that have been used for such a long time be scientifically supported for their efficacy.

**Limitations of traditional medicine**

Although traditional medicine is widely used in many diseases ailments, it has some limitations. The main limitation of traditional remedies is the lack of standardisation of raw materials, processing methods and plant products, dosage formulation and the non existence of criteria for quality control.

**Herbal Medicine –WHO guidelines**

The World Health Organisation is now actively encouraging developing countries to use herbal medicine which they have been traditionally used for centuries. The WHO has estimated that nearly 80% of the population of developing countries meet their primary health care needs through plant based traditional medicine. Global estimates indicate that over 75% of world population cannot afford the products of Western pharmaceutical industry and have to rely upon the use of herbal medicines that are derived from plants [3].

**Herbal Medicine - A Global View**

Tribal societies throughout the world have their respective system of medicine, using locally available plants as ingredient of medicine. The efficacy of plant drugs and plant derived wonder drugs with miraculous therapeutic effects and rich ethno-pharmaceutical uses are known from the tribes. Medicinal plants have been used for healing and preventive health for thousands of all around the world. For example the therapeutic efficacies of the following herbs well known worldwide (Korach 2011) such as *Aloe vera,* Aswagandha, Ginger, *Ginkgo biloba*, Ginseng root, Liquorice and Valerian. Research workers and industrialists engaged in the field of medicinal and aromatic plants have increased many folds and considerable efforts are being made all over the world to utilize more and more plant resources for the benefit of mankind [4].

**Factors responsible for herbal medicine used as herbal remedies**

Most of the population relies heavily on the use of herbal remedies because of their effectiveness, easy availability, low cost, inadequate supply of modern drugs, prohibitive cost of modern treatments, side effects of several allopathic drugs and shortage of practitioners of modern medicine in rural areas and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [9].

**Plants as a source of medicine**

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. The world is now moving towards the herbal medicine or

phytomedicines that repair and strengthen the bodily systems (especially the immune system, which can then properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects. For instance Chinese drug Mahung was in use for more than 5,000 years for the treatment of different types of fever and respiratory disorders. In spite of tremendous development in the field of synthetic drugs and antibiotics during the 21$^{st}$ century, plants still contribute one of the major sources of drugs in modern as well as traditional medicine throughout the world.

**Use of plants in modern medicine**

Many drugs of modern medicine have their origin in traditional medicine. Some common examples include the discovery of the alkaloid diosgenin in *Dioscorea deltoidea* used as source for the partial synthesis of cortisone and steroid hormones in the forties, the discovery of the hypotensive alkaloid reserpine in *Rauvolfia serpentina* and the analgesic alkaloid aspirin in *Filipendula ulmariain* in the fifties, the discovery of anti-asthmatic alkaloid ephedrine in *Ephedra sinica* and the anti-cancer alkaloid podophyllotoxin in *Podophyllum hexandrum* in the sixties [5].

**Natural products role in drug discovery**

Most of the natural products are compounds biosynthetically derived from primary metabolites and are generally categorized as secondary metabolites. These are the major sources of pharmaceutical industry to discover newer drugs. The reason for interest in natural product chemistry is serve as lead compound for newer drugs, give as much information on possible mechanism and thus on the molecular origin and basis of diseases (biotechnology). Natural products are permanent challenges with respect to total synthesis and stimulate the development of new reagents and reactions [6].

**Herbal products in Modern medicine**

In spite of tremendous advances made in the modern system of medicine these are still certain disease conditions for which suitable drugs are not available in the allopathic system of medicine. Moreover the modern system of medicine produces lot of side effects to the patients. And thus, new drugs are urgently needed for the cure of diseases, viral infections, liver diseases, diabetes, bronchial asthma, cancer, immunomodulatory as adjuvant for chemotherapy and adaptogens. Nowadays about 25-30% of today's prescription drugs contain chemicals derived from plants. Some 119 chemical constituents from 91 plants are now used in Western countries [7].

**Plants as important source for new drugs**

Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity or reduced toxicity. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments [8].

On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. The active molecule when it cannot be synthesised economically, must be obtained from the cultivation of plant material.

The scientific study of traditional medicines, derivation of drugs through bio-prospecting and systematic conservation of the concerned medicinal plants are thus of great importance today [9].

Hence, an attempt is made to study the medicinal properties of a little known twinning herb namely *Ipomoea pes-tigridis,* on which very little work has been carried out so far. The aim of the study is to add this herb into the herbal formulary.

# CHAPTER-2

## REVIEW OF LITERATURE

The chapter provides retrieval of papers both from primary and secondary sources. The literature review encompasses information of systematic position, pharmacognosy, biological studies and phytochemistry of *Ipomoea pes-tigridis* and other *Ipomoea* species. It was found that very little information was available for *Ipomoea pes-tigridis* as compared to voluminous work other species of *Ipomoea.* Paucity of information for *Ipomoea pes-tigridis* formed the rationale for the present study.

***Ipomoea pes –tigridis***

**Shukla G and Verma BK (1990)** have described the taxonomic status of *Ipomoea batatas, Ipomoea carica, Ipomoea hederifolia, Ipomoea aquatia, Ipomoea pes-tigridis, Ipomoea turbinata, Ipomoea carnea, Ipomoea obscura, Ipomoea sepiaria* and *Iopomea sinensis* [10].

**Singh V and Gupta PC (1995)** have isolated a water soluble seed gum polysaccharide containing D-galactose and D-mannose as sugars from *Ipomoea pes-tigridis* and its structure elucidation has been described [11].

**Azam MM and Sherwani MRK (1999)** have investigated the fatty acid composition of seed oils collected from the seeds of *Ipomoea pes-tigridis* and some other genus in arid zone of Rajasthan. The seed oils were investigated by using GC/MS technique [12].

**Sharma DP (2002)** has studied the nutritional qualities of *Ipomoea pes-tigridis*. The plant was highly nutritive with peak of 4195.20 cal/g energy and 24.25% crude protein in October & 0.196% phosphorous content in September and 5.8% potassium content in November [13].

**Other species**

**Pharmacognosy**

**Parveen F and Bhandari MM (1982)** have discussed the polynogical account of 7 genera and 23 species of Convolvulaceae found in Indian desert and pollen morphology in relation to taxonomy of the family [14].

**Karatela YY and Gill LS (1985)** have described the epidermal morphology and stomatal ontogency of ten plant (Convolvulaceae) species. The leaves of the plants shows amphistomatic, paracytic stomata type with eumesogenous ontogeny in all the Convolvulaceous species except in two *Ipomea* species [15].

**Siddiqi TO *et al.*, (1990)** have identified the botanical character of Kaladana (*Ipomoea hederarea*) [16].

**Phytochemistry**

**Sen M *et al.*, (1979)** have investigated the whole plant *Gerbera nuginosa.* Taraxerol and taraxecyl acetate were isolated from the benzene as well as petroleum ether extract of the plant. β-sitosterol was also isolated from the leaves *of Ipomoea fistulosa* [17].

**Goto T *et al.*, (1981)** have reported the structure of an alkali hydrolysis product of heavenly blue anthocyanin (HBA) which was isolated from blue flowers of morning glory *(Ipomoea spp),*and it was determined to be trans-o–[6-o-(-trans-*3*-*o*-β-D—caffeyl)-β-D-glucopyronosyl] caffeic acid by spectral studies [18].

**Takagi S *et al.*, (1981)** have isolated the isoquercitrin monoacetate, isoquercitrin hyperin and quercetin sodium salt from *Ipomoea biloba* by column chromatography and were identified by thin layer chromatography [19].

**Dubey P *et al.*, (1982)** have described the isolation of a new flavanoid from ethyl acetate extract of *Ipomoea fistulosa* and its structure was elucidated by spectral studies [20].

**Schneider JA et al., (1984)** have isolated nine new sesquiterpenes from the root of *Ipomoea batatas* and their structures were also determined [21].

***Harrison DA and Kulshreshtha DK (1986)*** have examined the four new fatty acid glycosides from *Ipomoea dichroa*. They were identified as dichrosides A, B, C and D besides friedelin, stearic acid, β-sitosterol and its glucosides [22].

**Wilkinson RE *et al.*, (1987)** have isolated the seed alkaloids from *Ipomoea hederifolia, Ipomoea coccinea, Ipomoea wrightii* and these alkaloids were quantified by spectrophotometry as ergonovine maleate equivalents [23].

**Ono M *et al.*, (1991)** have isolated the ether soluble resin glycosides, operculins from the roots of *Ipomoea operculata.* They were characterized on the basis of chemical and spectral analysis [24].

**Ysrael MC and Waterman PG (1994)** have evaluated the isolation of antifungal and cytotoxic principles from *Ipomoea muricata* [25].

**Yahara S *et al.*, (2002)** have isolated the phenolic compounds from the methanolic extract of dried leaves of *Ipomoea batatas* and these compounds were identified by spectral studies [26].

**Pareda-Mirinda R *et al.*, (2005)** have suggested the hexane soluble extract from the aerial parts of the herbal drug *Ipomoea pes-caprae* beach (morning glory) through preparative scale recycling HPLCwhich yielded six lipophilic glycosides [27].

**Sing V *et al.*, (2007)** have isolated a non ionic water soluble galactomannan (galactose:mannose in 1:6 molor ratio) from endosperm of the seeds of *Ipomoea dasysperma*. The seed gum possesses non ionic characteristics of commercial seed gum and has the potential to be used in food and pharmaceutical industry. [28]

**Pharmacology**

**Kappor A *et al.*, (1981)** have demonstrated the petal extracts of *Ipomoea carnea, Ipomoea palmate* exhibit antifungal activity against *Alternarria brassicae, Alternaria brassicicola* and *Fusarium oxysporium* due to presence of some flavonoids [29].

**Khare AK *et al.*, (1982)** have evaluated the anti-inflammatory activity of *Ipomoea turpethinum*. The aqueous extract of root showed more potent activity (38.3) against acute, subacute and chronic inflammation in carageenan induced paw edema model compared to alcoholic (32%) and ethereal extract (26%) [30].

**Mujumdar AM *et al.*, (1983)** have demonstrated that the ethanol extracts of *Ipomoea leari* seeds produced depression at low doses and stimulation at higher doses, ultimately which lead to convulsions in mice and at higher doses analgesic activity was noted. The antibacterial activity was also studied [31].

**Dasilvo Filho AA *et al.*, (1986)** have described the isolation of four new antimicrobial glycosides from *Ipomoea bahensis*. They showed significant activity against sarcoma 180 in mice [32].

**Pongprayoon U (1991)** has studied the effect of extract of *Ipomoea pes-caprae* in the treatment of dermatitis caused by poisonous jelly fish toxins. It also exhibited significant antispasmodic activity in isolated guinea pig ileum and anti-inflammatory in carageenan induced in rat paw edema [33].

**Reza MS *et al.*, (1994)** have studied the antibacterial activity of the chloroform extract of different parts of *Ipomoea fistulosa* against all the strains of *Shigella* and the gram positive bacteria namely *Bacillus megaterium* and *Bacillus polymyxa*. The extract exhibited significant activity [34].

**Reynolds WF *et al.*, (1995)** have isolated and characterized the cytotonic and antibacterial tetrasaccharide glycoside from *Ipomoea stans*. These compounds have

pronounced cytotoxicity towards 3 human tumor cell lines as well as specific antibiotic activity against two bacterial strains [35].

**Navarro-Ruiz A *et al.*, (1996)** have demonstrated the anti-convulsant effect of aqueous, hydroalcoholic and chloroform extracts from roots of *Ipomoea stans*. Maximal electroshock seizure inducing test (MES) and subcutaneously injected metrazole (METSC) were the *premental epilepsy* model used and maximum activity was seen with aqueous extract [36].

**Matsui T *et al.*, (2002)** have performed anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas*. They suggested that anthocyanin had alpha glucosidase inhibitory activity after a single oral administration in 8week old male rats [37].

**Barnes CC *et al.*, (2003)** have reported that the organic soluble extract from the leaves of *Ipomoea leptophylla* showed activity against *M. tuberculosis (Invitro method*) [38].

**Dhembare AJ and Sangle S (2003)** evaluated the effect of various plant extracts (*Piper nigrum, Ipomoea fistulosa*) for their antimicrobial activity against human pathogenic bacterial strains [39].

**Haueza IM *et al.*, (2003)** have reported the immunomodulatory activity of *Ipomoea carnea* on peritoneal cells of rat model. The experimental model suggested that low doses of *Ipomoea carnea* induced enhanced phagocytosis activity and hydrogen peroxide production by macrophages [40].

**Yoshimoto M *et al.*, (2004)** have estimated the phenolic composition and radical scavenging activity of sweet potato *Ipomoea batatas* treated with Koji [*Aspergillus awamori* and cellulase (cellulosin T2)] [41].

**Prasad KN *et al.*, (2006)** have studied callus induction from *Ipomoea aquatica* Forsk leaf and its antioxidant activity. The antioxidant activity was analysed by DPPH, TBARS and

metal chelating methods. Hyper antioxidant activity was observed in one month old callus produced by NAA in combination with Kinetin [42].

**Ferreira AA *et al.,* (2006)** have evaluated and anti-nociceptive effect from *Ipomoea cairi*ca. The ethanolic extract (100, 300, 1000 and 3000mg/kg; per oral) of *Ipomoea cairica* induced dose dependent reduction of response in the formalin test inflammatory phase in mice model. The same dose range did not modify neurogenic pain in formalin test, tail flick reflex latency, in carageenan induced paw edema and rota-rod test motor performed [43].

**Datta Choudry M *et al*., (2007)** have studied the chemical characterization and antifungal, CNS depressant activity of *Ipomoea aquatica*. The methanolic extract of *Ipomoea aquatica* showed the antifungal activity against *Candida albicans* (1.6cm) and for CNS depressant activity in mice which was near to the effect of standard [44].

**Sokeng SD *et al.,* (2007)** have demonstrated the inhibitory effect of *Ipomoea aquatica* extracts on glucose absorption using a perfused rat intestinal preparation model. The aqueous/dichloromethane/methanol extracts showed the significant inhibitory effect on glucose absorption in test animals. The most pronounced effect was observed with the aqueous extract. Quabain was used has a reference inhibitor [45].

**Hamsa TB and Kuttan G (2007)** have reported the antioxidant activity of methanolic extract of *Ipomoea obscura. In vitro* model, the extract was found to have potential antioxidant activity as it inhibited lipid peroxidation, scavenge hydroxyl, superoxide and nitric oxide radicals generated. *In vivo* model, the extract was found to inhibit the PMA induced superoxide generation in mice peritoneal macrophages [46].

**Vimala Y *et al.,* (2007)** have demonstrated the antimicrobial activity of *Ipomoea kentrochulos* on microorganisms and isolates. The MIC of crude residual extract determined by *tube dilution* against different microbes was more than 800µg/ml [47].

**Rivera IL *et al.*, (2008)** have studied the anti-mycobacterial activity, cytotoxicity and effects on the central nervous system of *Ipomoea tyrianthina* [48].

**Chimkode R *et al.*, (2009)** have evaluated the wound healing activity of tuberous root extracts of *Ipomoea batatas in vivo model*. The petroleum ether extract showed more potent wound healing activity (dead space granulation) models compared to other extracts [49].

# CHAPTER-3

## AIM AND SCOPE OF THE PRESENT STUDY

Today there is a need for scientific validation of plants that has been used in folklore medicine because of loss of information, advancement of therapy and deforestation that is taking place. The aim of present study is to scientifically validate *Ipomoea pes-tigridis*, a twining herb which is being used as a fodder for animals.

The ethnomedical information of the plant reveals that the herb *Ipomoea pes-tigridis* Linn. was used as an antidote to dogbite, boils and carbuncles, the leaves were applied as a poultices for boils, carbuncles and sores. The root of this plant was found to possess purgative action. The phytochemical studies on the leaves have been reported for the presence of carbohydrates, proteins and resins [50].

Hence the present work has been designed to carry out the following studies on the leaves *Ipomoea pes-tigridis* Linn.

1. Pharmacognostical studies on the leaves.
2. Preliminary phytochemical screening on the extracts
3. Quantitative analysis of phyto constituents
4. Estimation of total phenolics and total flavanoids.
5. Phytochemical evaluation of extract of *Ipomoea pes-tigridis* by TLC and HPTLC studies.
6. Screening of the 70% ethanolic extract of leaves for following pharmacological activities.
    - *In vitro* antioxidant activity
        - DPPH radical scavenging activity
        - Nitric oxide scavenging activity

- Reducing power assay
- Hydrogen peroxide scavenging activity

- *In vitro* anti-inflammatory activity (HRBC membrane stabilisation method)
- *Invitro* anti-arthritic activity (Protein denaturation method) Cytototoxicity studies
- *In vitro* anticancer activity on liver HEPG2 cell lines by MTT assay
- Anti-bacterial activity

# CHAPTER-4

## PLANT PROFILE [51-53]

**BIOLOGICAL SOURCE** **:** *Ipomoea pes-tigridis* Linn.

**FAMI LY** **:** Convolulaceae

**SYSTEMATIC POSITION**

| | |
|---|---|
| Subkingdom | : Tracheobionta |
| Super division | : Spermatophyta |
| Division | : Magnoliophyta |
| Class | : Magnoliopsida |
| Subclass | : Asteridae |
| Order | : Solanales |
| Family | : Convolvulaceae |
| Genus | : *Ipomoea* L. |
| Species | : *pes-tigridis L.* |

**VERNACULAR NAMES [51]**

| | |
|---|---|
| English | : Morning glory, Tiger foot |
| Hindi | : Ghiabati, Panch-patri |
| Malayalam | : Pulichuvadu |
| Sanskrit | : Arishta, Shankine. |
| Tamil | : Pulichovadi, Punaikkirai. |
| Telugu | : Chikunuvvu, Mekama-dugu, Puritikada. |
| Bengali | : Langulilata. |
| Oriya | : Bilaipado |

## GEOGRAPHICAL DISTRIBUTION [52]

The plant is found more or less throughout India usually in hedges, grasslands, waste places, bushes, fields and seacoast. In North India, it grows profusely during the monsoon and remains green and succulent for 3-4 months. It is also distributed in tropical E. Africa and tropical Asia.

## HABIT AND HABITAT

*Ipomoea pes-tigridis* is a spreading or twinning herb. It is herbaceous annual, almost throughout India ascending up to 4000 ft, plains from the coast, to 750-900m, often in arable lands. The climber flowers throughout the year.

## ETHNOMEDICINAL USES [53]

1. The herb is used in the treatment of boils, carbuncles and as antidote to dog bites.
2. In Philippines and Indonesia, the leaves are applied as poultices to boils, pimples and sores. The root is used as a purgative.

# CHAPTER - 5

## PHARMACOGNOSTICAL STUDIES

## MATERIALS AND METHODS

### Morphological studies

The plant specimens were collected from Madurai Medical College campus. The plant was identified and authenticated by Dr. L. Stephen, Lecturer, American College and also by Dr. John Britto, Rapinet Herabarium, St. Joseph's College, Tiruchirapalli. The authenticated herbarium sheet has been placed at the Dept. of Pharmacognosy, College of Pharmacy, Madurai Medical College. A copy of the herbarium has been presented in **Fig. 1.** A special care was taken to select healthy plants and normal organs for macroscopical studies. The macroscopical features of the plant are presented in **Fig. 2.**

### Microscopical Studies of the leaves of *Ipomoea pes-tigridis*

### Collection of Specimen

The samples of the leaf were cut and removed from the plant and fixed in FAA (formalin-5ml + acetic acid-5ml + 70% ethyl alcohol-90ml). After 24h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass, 1940. The infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were then cast into paraffin blocks.

### Sectioning

The paraffin embedded specimen was sectioned with the help of rotary microtome. The thickness of the section was 10-12µm, de-waxing of the section was carried out by customary procedure [54]. The sections were then stained with toludine blue as per the method published by O'Brien *et al.* [55]. Since toludine blue is a polychromatic stain, the

staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. The sections were also stained with saffranin and fast-green and iodine wherever necessary.

For studying the stomata morphology, venation pattern and the trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) was used. The clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [56] were also prepared for studying stomatal morphology. Glycerin mounted temporary preparations were made for macerated/ cleared material.

**Powder microscopy**

The powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

**Photomicrographs**

The photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. The magnification of the figures was indicated by the scale bars on the photomicrographs. The microscopic features observed for the leaves of the plant are presented in **Fig. 3 to 11.**

**QUANTITATIVE MEASUREMENTS [57]**

**Determination of Leaf Constants**

The stomatal number, stomatal index, vein islet number and vein termination number were determined on fresh leaves by using standard procedures.

**Determination of Stomatal Number**

The **stomatal number** may be defined as the "average number of stomata per square mm area of epidermis of the leaf".

Small pieces of upper and lower epidermal peelings of the leaves were mounted onto a slide. The camera Lucida and stage micrometer were used to draw 1mm square on a paper. The stage micrometer was replaced by the preparation slide. Then the preparations were observed under microscope and the stomata were marked in that unit area. The number of stomata present in unit area was calculated. Six such readings were taken and the average of stomatal number was calculated and presented in the **Table 1** for both upper and lower epidermis.

**Determination of Stomatal Index**

The **stomotal index** is the percentage of the ratio of the numbers of stomata to the total number of epidermal cells where each stoma also being counted as one cell. It is calculated using the following formula **S.I** $= \frac{S}{E+S} \times 100$**; w**here S is the number of stomata per unit area and E is the number of epidermal cells in the same unit area

The procedure adopted for the determination of stomatal number was followed and the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula and was given in **Table 1.**

**Determination of vein islet number and vein termination number**

The term vein islet is used to denote the minute area of photo synthetic tissue encircled by the ultimate division of the conducting strands. The **vein islet number** may be defined as the" number of vein islets per square mm area".

The term **vein termination number** may be defined as the "number of vein terminations present in one square mm area of the photosynthetic tissue".

Small pieces of leaves were cut on the lamina between midrib and the margin, cleared in chloral hydrate and mounted on a slide. The camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1mm square was drawn on the paper. Then the stage micrometer was replaced by the sample slides and the veins were traced over the square. The vein islets and vein terminations were counted in the square. Six such readings were taken and the average was calculated and the results were presented in **Table 1.**

**Physical Parameters [58-61]**

**Determination of Volatile Oil [59]**

Volatile oils are characterized by their odour, oil like appearance and also it has the ability to volatilize at room temperature. Chemically they are mixtures of monoterpenes, sesquiterpenes and their oxygenated derivatives. Volatile oils can be estimated by hydro distillation method.

An accurately weighed 100g of plant material was crushed and introduced in to the flask containing distilled water until one third of the plant material was immersed and few pieces of porcelain bits were added. The flask containing liquid was heated until it boils. After 3h, heating was stopped and the volume of the collected oil was recorded on the graduated receiver tube. The oil content of the plant material was calculated in mL/100g of plant material. The result obtained was presented in **Table 2.**

**Determination of foreign organic matter [58]**

The part of organ or organs other than those specified in the definition or description of the crude drugs is defined as foreign organic matter. An accurately weighed 100g of air dried coarse drug and spread out in a thin layer. The sample drug was inspected with the unaided eye or with the use of 6X lens and the foreign organic matter was separated manually as completely as possible and weighed. The percentage of foreign organic matter was calculated with reference to the weight of the drug taken. The result is presented in **Table 2.**

**Determination of Ash Values [58, 61]**

The ash values were determined by using air dried powdered leaves as per the official method.

**Total ash**

An accurately weighed crude leaf powder (2g) was transferred into a tared nickel crucible. The ground drug was scattered in a fine even layer on the bottom of the crucible and incinerated by gradually increasing the heat not exceeding 450˚C (dull red heat) until free form carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried drug was calculated. The procedure was repeated in triplicate. The values are presented in **Table 2.**

**Acid insoluble ash**

The ash obtained for total ash was boiled for 5min with 25mL of 2M hydrochloric acid. The insoluble matter was collected in a tared sintered glass crucible. The residue was washed with hot water, ignited to constant weight, cooled in a desiccator and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated. The procedure was repeated in triplicate. The values are presented in **Table 2.**

**Water soluble ash**

The ash obtained for total ash was boiled with 25mL of distilled water. The insoluble matter was collected in a Gooch crucible, washed with hot water ignited to a constant weight and cooled in a desiccator and weighed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference gave the weight of the water soluble ash. It was calculated with reference to the air-dried powder. The results were represented in **Table 2.**

**Loss on drying [58]**

About 2g of the powdered crude drug was accurately weighted in a tared dish and dried in an oven at 100˚-105˚C. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken and the results obtained are presented in **Table 2.**

**Determination of Foaming Index [61]**

Plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. "The foaming ability of an aqueous decoction of plant materials and their extracts" is measured in terms of foaming index.

An accurately weighed 1g of the coarse plant material was transferred into a 500mL conical flask containing 100mL of boiling water. The flask was maintained at moderate boiling for 30min. The solution was cooled and filtered into a 100mL volumetric flask and sufficient distilled water was added to dilute to volume. The decoction was poured into 10 stoppered test tubes in successive portions of 1mL, 2mL, etc. upto 10mL, and the volume of the liquid in each tube was adjusted with water upto 10mL. The tubes were stoppered and shaken in a length wise motion for 15sec (two shakes/sec) and allowed to stand for 15min. The height of the foam was measured. If the height of the foam in every tube was less than

1cm the foaming index was less than 100. If a height of foam of 1cm was measured in any test tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If this tube was first or second tube in a series, an intermediate dilution was prepared in a similar manner to obtain a more precise result.

If the height of the foam was more than 1cm in every tube, the foaming index was over 1000. In this case, the determination was repeated using a new series dilution of the decoction in order to obtain a result. The foaming index was calculated using the following formula 1000/A where A was the volume in mL of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm was observed. The result obtained is presented in **Table 2**.

**Determination of Swelling Index [61]**

Swelling index is the "volume in mL taken up by the swelling of 1g of plant material under specified conditions". Its determination is based on the addition of water. Using a glass stoppered measuring cylinder, the material is shaken repeatedly for 1h and then allowed to stand for required period of time. The volume of the mixture (in ml) is read.

An weighed 1g of powder was introduced in to a 25mL glass stoppered measuring cylinder and 25mL of water was added and the mixture was shaken thoroughly every 10min for 1h and allowed to stand for 3hrs at room temperature. The volume in mL occupied by the plant material including the sticky mucilage was measured. The mean value of the individual determination was calculated related 1gm of plant material. The result is tabulated in **Table 2**

**Determination of Extractive values [61]**

**Petroleum ether soluble extractive value**

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of petroleum ether in a closed flask for 24h, shaking frequently during 6h and allowed

to stand for 18h. It was filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the petroleum ether soluble extractive with reference to the air dried drug was calculated and presented in **Table 3.**

**Ethanol soluble extractive**

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of ethanol in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h. It was filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the ethanol soluble extractive with reference to the air dried drug was calculated and presented in **Table 3.**

**Water-Soluble Extractive**

An accurately weighed 5g of coarsely powdered air dried drug was macerated in 100mL of chloroform water in a closed flask for 24h, shaking frequently during 6h and allowed to stand for 18h. It was filtered rapidly taking precautions against loss of solvent. 25mL of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105˚C to constant weight. The percentage of the water soluble extractive with reference to the air dried drug was calculated and presented in **Table 3.**

**Chloroform, Acetone, Methanol and hexane soluble extractives**

The procedure followed for the determination of alcohol soluble extractive value was adopted for the determination of chloroform soluble extractive, acetone soluble extractive, methanol soluble extractive and hexane soluble extractive. Instead of ethanol respective solvents were used for the determination of their extractive values.

The percentage of chloroform, acetone, methanol and hexane soluble extractives were calculated and presented in **Table 3.**

**Powder analysis**

The behaviour of the powder with different chemical reagents was carried out as mentioned by Kay 1938 [62] and Johansen 1940 [54]. The observations are presented in **Tables 4 & 5**.

**Fluorescence analysis**

The fluorescent analysis of the drug powder as well as the plant extracts of *Ipomea pes-tigritis* were carried out by using the method of Chase and Pratt 1949 [63]. The observations are tabulated in **Tables 5 & 6.**

## RESULTS AND DISCUSSION

### MACROSCOPIC FEATURES

**Leaves (Fig. 2.1, 2, 7, 8)**

The leaves are palmately 5-9 lobed and divided below the middle. They are transversely elliptic in outline, 3-13 cm length, oblong, tapered at both ends, densely pubescent, apex mucronate, branchlets hispid. The lamina is chartaceous. The petiole is 8 cm long.

**Flowers (Fig.2.4)**

The flowers are aggregated into capitates clusters with 2-18 cm long peduncle, linear to oblong bract in 1-3cm long. The sepals are five lobed, the lobes are slightly unequal with 7-12 mm long. The corolla is white or light pink and funnel shaped with unequal filaments. Stamens 5, epipetalous hairless ovaries which are conical in shape are present.

**Fruits (Fig. 2.5, 6)**

Capsules are globose, 0.8cm across hairs in small tuffs. The capsule is 7 mm long, 4-valved. The seeds are ellipsoid, pubescent and gray tomentellous.

### MICROSCOPICAL CHARACTERS

**Leaf (Fig. 3)**

The leaf consists of a thick, abaxially hanging midrib (**Fig.3.1).** It is 500µm thick and 700µm wide. It consists of distinct, fairly thick squarish epidermal cells with thin walls. The ground tissue is homogenous and parenchymatous; the cells are angular thin walled and compact. The vascular strand is single bowl shaped and bicollateral. It consists of several (about 11) short lines of xylem elements intermixed with sclerenchymatous ground tissue. The xylem elements are circular, thick walled and lignified. Many small nests of phloem occur along the lower and upper surfaces of the xylem strands (**Fig. 3.2)**.

The lateral veins are thin and do not project much beyond the level of the lamina (**Fig. 4. 1).** The vascular strand of the lateral veins consists of two to four short compact lines of xylem elements and small nest of phloem elements. The vascular strand is surrounded by dilated hyaline bundle sheath cells (**Fig. 4.1).**

**Lamina (Fig. 4)**

The lamina is 700µm thick **(Fig. 4.2).** The adaxial epidermis consists of thick rectangular or squarish cells with thin walls. The abaxial epidermis is comparatively thin; the cells being circular to rectangular. The mesophyll is differentiated in to thick adaxial band of single row of compact cylindrical palisade cells and abaxial zone of three or four layers of lobed, loosely arranged spongy parenchyma cells.

Calcium oxalate crystals of druses or sphaero crystals are common in the mesophyll tissue (**Fig. 4.3).** The druses are located in the spongy parenchyma. The druses vary in size from 20-40µm.

**Epidermal cells and stomatal type (Fig .5)**

The epidermal cells and stomata were studied in paradermal sections of the leaf-blade. The epidermal cells are wide with thick, wavy anticlinal walls. The cells appear amoeboid in outline. Cuticular markings are warting. The stomata are predominantly paracytic type **(Fig. 5.2).** The stoma has two subsidiary cells, one on either side or parallel to the guard cells. The subsidiary cells may be equal or unequal. The stomata are broadly elliptical with slit like stomatal pore. The stomata are 15×20µm in size. The stomatal number is 48/mm².

**Venation pattern (Fig. 6)**

The lateral veins are thin and straight and form reticulate venation. The vein-islets are variable in size and shape. Some of the islets are rectangular, some are 5 sided and others are polygonal. The vein terminations are well developed. They are either forked once or unbranched. The terminations are curved and are undulate **(Fig. 6.2).**

**Petiole (Fig. 7)**

Both distal (terminal) and proximal (lower) parts of the petiole were studied. The distal part of the petiole is semi circular along the abaxial side and the adaxial side has median shallow groove (**Fig. 7.1).** It is 1mm thick and 2mm wide. The epidermal layer is made up of distinct squarish thin walled cells. The ground tissue is parenchymatous and homogenous, the cells being circular, thin walled and compact.

The vascular system is multi stranded **(Fig. 7.1).** There is an arc of three vascular segments at the abaxial part and two in the wing part of the midrib, one in each corner (**Fig. 7.1)**. All the strands are bicollateral having phloem elements both on the outer and inner sides of the xylem strands. The xylem elements are in short radial rows; the elements are circular and thin walled **(Fig.7.2).**

**Distal part of the petiole (Fig. 8, 9)**

The distal part of the petiole is thick, circular and adaxially grooved (**Fig.8.1).** It is 3.7mm thick and 2.4mm wide. It consists of thin epidermal layer of small thin walled cells, outer narrow zone of collenchyma cells and parenchymatous ground tissue. The vascular system includes a broad and thick bowl shaped lower vascular strand and two small segments of wing strands. The vascular strand on the abaxial part includes several fairly long uniseriate rows of xylem elements. The wing bundles also have limited number of short rows of xylem elements. Phloem occurs on both sides of the xylem in small groups **(Fig. 8, 9.1).** Calcium oxalate druses are seen in the ground cells inner to the epidermis **(Fig. 9.2).** The druses are up to 20µm wide.

**Powder microscopy characters (Fig. 10 & 11)**

The leaf powder shows the following components when examined under the microscope.

**Epidermal Trichomes (Fig. 10)**

Nonglandular epidermal trichomes are abundant on the lamina. The trichomes are unicellular and unbranched. They are uniform in thickness and gradually tapers into pointed tip. The walls are thick and the cell lumen is narrow. The trichomes are 850µm to 1.25mm long and 30µm thick.

**Glandular Trichomes (Fig. 11)**

Subsessile glandular trichomes are common in the powder. The glands are seen detached from the epidermis. They are peltate-type. The body of the trichome is circular and multicellular comprising 6-12 triangular radiating cells with dense cell contents. The glands are 50-140µm in diameter.

**QUANTITATIVE MEASUREMENTS**

**Determination of leaf constants**

The values results obtained quantitative microscopical parameters are presented in **Table 1.** From the results obtained, it can be seen that the number of stomato present in upper epidermis was 34 ± 0.27 while in lower epidermis it was 48 ± 0.75. The vein islet number was found to be 5.0 ± 0.54 and the vein termination was found to be 23.1 ± 0.54.

**Table 1: Quantitative microscopical parameters of the leaf of *Ipomoea pes-tigridis* Linn**

| S. No. | Parameters* | Values obtained* |
|---|---|---|
| 1 | Stomatal number in upper epidermis | 34± 0.27 |
| 2. | Stomatal number in lower epidermis | 48 ± 0.75 |
| 3. | Stomatal index in upper epidermis | 32.23± 0.46 |
| 4. | Stomatal index in lower epidermis | 47.78 ± 0.22 |
| 5. | Vein islet number | 5.0 ± 0.54 |
| 6 | Vein termination number | 23.1 ± 0.54 |

***Mean of 6 readings ± SEM**

The determination of leaf constant will help in determination of identity of the plant. These parameters also help in distinguishing the species from other species of the genus since these characters are always fall within the range observed.

**Determination of the physical constants**

The results obtained for the physical constants like volatile oil, loss on drying, ash values, swelling index etc. are presented in **Table 2.** From the **Table 2,** it can be observed that the total ash obtained was nearly 10.75% while loss on drying was found to be 2.72 ± 0.11. The acid insoluble ash was 0.25% of the crude material and hence can be said that no earthy matter is adherent to the leaves of the plant. The water soluble ash was found to be 6.43 ± 0.08. These values help in identifying whether the material has already been exhausted when available in powdered form and also helps in the determination of purity of the crude drug.

**Table 2: Analytical parameters of *Ipomoea pes-tigridis* Linn**

| S. No | Parameters | Values expressed in % |
|---|---|---|
| **1** | **Volatile oil** | Nil |
| **2** | **Foreign Organic Matter** | 0.02 ±0.01 |
| **3** | **Ash values** | |
| | Total ash | 10.75 ± 0.08 |
| | Water soluble ash | 6.43 ± 0.08 |
| | Acid insoluble ash | 0.25 ± 0.01 |
| **4** | **Loss on drying** | 2.72 ± 0.11 |
| **5** | **Foaming Index** | Less than 100 |
| **6** | **Swelling Index** | Initial volume 2.08 ± 0.09<br>Final volume 9.78 ± 0.03 |

*** mean of three readings ±SEM**

The values obtained for extraction with various solvents are presented in **Table 3.** From the **Table 3,** it can be seen that the 70% ethanol and water showed a maximum percentage extractive value of 28.08 ± 0.12 and 22.50 ± 0.05 and hence 70% ethanol was

used an extracting menstrum for extracting active principles for phytochemical and pharmacological evaluation.

**Table 3: Extractive values of *Ipomoea pes-tigridis* Linn**

| S. No | Extractive values | Values* expressed as % |
|---|---|---|
| 1 | Petroleum ether extract | 0.40 ± 0.02 |
| 2 | Hexane | 0.96 ± 0.03 |
| 3 | Chloroform | 0.88 ± 0.01 |
| 4 | Acetone | 2.16 ± 0.02 |
| 5 | Ethyl acetate | 3.60 ± 0.05 |
| 6 | Methanol | 18.96 ± 0.22 |
| 7 | Ethanol | 15.24 ± 0.18 |
| 8 | 70% Ethanol | 28.08 ± 0.12 |
| 9 | Aqueous | 22.50 ± 0.05 |

* **Mean of three readings ± (SEM)**

**Powder analysis**

The behaviour of the powder with various organic reagents and compounds are presented in **Table 4.** The powder analysis revealed the presence of phytosterols, tannins, proteins, flavanoids and phenolic compounds when seen by the naked eye after treating them the reagents.

**Table 4: Behavior of the *Ipomoea pes-tigridis* powder with various chemical reagents**

| Powder + Reagents | Colour / Precipitate | Presence of active principle |
|---|---|---|
| Picric acid | Yellow precipitate | Protein present |
| Conc. sulfuric acid | Reddish brown color | Phyto sterols present |
| Lieberman Burchard reagent | Reddish brown color | Phyto sterols present |
| Aqueous ferric chloride | Greenish black color | Tannins present |
| Iodine solution | Blue color | Starch present |
| Mayer's reagent | No Cream color | Alkaloids absent |
| Spot test | No stain | Fixed oils absent |
| Sulfosalicylic acid | White precipitate | Protein present |
| Aqueous sodium hydroxide | Yellow color | Flavanoids present |
| Mg – HCl | Magenta color | Flavanoids present |
| Aqueous lead acetate | White precipitate | Presence of tannins |

**Note: - Colour reactions are viewed under natural light by naked eye**

**Fluorescence analysis**

The results obtained for fluorescence analysis of the powder and the various extracts of *Ipomoea pes-trigridis* are presented in **Tables 5 & 6.** The powder when viewed at 254nm and 365nm after treatment with aqueous sodium hydroxide appeared green and black respectively. The powder when viewed with iodine at 254nm appeared dark green while at 366nm it appeared violet.

**Table 5: Fluorescence Analysis of powder of *Ipomoea pes-tigridis* Linn**

| Powder +reagent | Day light | UV light (254 nm) | UV light (366 nm) |
|---|---|---|---|
| Drug powder | Green | Dark Green | Greenish black |
| Drug powder +aqueous 1M sodium hydroxide | Green | Greenish black | Black |
| Drug powder + alcoholic 1M sodium hydroxide | Green | Green | Black |
| Drug powder + iodine | Reddish brown | Dark Green | Violet |
| Drug powder + 10% potassium hydroxide | Pale green | Green | Black |
| Drug powder + 1M hydrochloric acid | Yellowish Green | Green | Black |
| Drug powder + glacial acetic acid | Yellowish Green | Green | Brownish black |
| Drug powder + 50% sulphuric acid | Green | Greenish black | Black |
| Drug powder + 50% nitric acid | Dark Green | Greenish black | Black |
| Drug powder + 50% hydrochloric acid | Dark Green | Greenish black | Black |

From the **Table 6,** it can be seen that the hydro alcoholic extract of *Ipomoea pes-tigridis* when seen in day light was yellowish green while under UV light it was blackish green. The aqueous extract was brown when seen in day light and black under UV light.

**Table 6: Fluorescence Analysis of extracts of *Ipomoea pes-tigridis* Linn.**

| Extracts | Consistency | Colour in Day Light | Colour under UV Lamp | |
|---|---|---|---|---|
| | | | 366nm | 254nm |
| Petroleum extract | Semisolid | Yellowish green | Violet | Green |
| Hexane extract | Semisolid | Greenish black | Black | Green |
| Chloroform extract | Semisolid | Greenish brown | Orange | Green |
| Ethyl acetate extract | Semisolid | Yellowish green | Greenish black | Green |
| Ethanol extract | Semisolid | Yellowish green | Blackish green | Green |
| Methanol extract | Semisolid | Yellowish green | Black | Green |
| Aqueous extract | Semisolid | Brown | Black | Blackish green |

The macroscopical, microscopical and other pharmacognostic studies of a plant helps in the botanical identity of the plant as a whole and also in the crude and powdered form. These parameters are important because improper identification of the plant leads to unwanted side effects, poisoning etc. when the drug is ingested in powdered form or in the form of a formulation.

# CHAPTER-6

## PHYTOCHEMICAL EVALUATION

### MATERIALS AND METHODS

#### Collection of plant material

The leaves of *Ipomoea pes-tigridis* were collected in Madurai Medical College Campus during the month of September and authenticated by a taxonomist. The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies.

#### Preparation of extract

About 500g of the dried powdered leaf of *Ipomoea pes-tigridis* was defatted with 1.5L petroleum ether (60-80$^0$ C) by maceration. The solvent was removed by filtration and the marc was dried. To the dried marc 1.5L of 70% ethanol was added and the extraction was performed by triple maceration (72h process). It was then filtered and the combined filtrate was evaporated to a cohesive mass using rota vapour.

#### Preliminary Phytochemical Screening [64-66]

#### 1. Test for sterols

The powdered leaf was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

**a. Salkowski's Test**

A few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turns red in color if there is a presence of sterols.

**b. Liebermann – Burchard's Test**

To the chloroform solution a few drops of acetic anhydride and 1mL of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring will be formed and the upper layer turns green if there are sterols.

**2. Test for terpenoids**

A little of the powdered leaf was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionylchloride. A pink solution will appear if there are terpenoids.

**3. Test for carbohydrates**

**a. Molisch's Test:**

The aqueous extract of the powdered leaf when treated with alcoholic solution of α-naphthol in the presence of sulphuric acid showed a purple colour if there are carbohydrates.

b. **Fehling's Test**:

The aqueous extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. A red precipitate will be obtained if there are free reducing sugars.

**4. Test for flavonoids**

**a. Shinoda's test**

A little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. A red colour will be obtained if there are flavonoids.

**b. Alkali Test**

To the small quantity of test solution, 10% aqueous sodium hydroxide solution was added. A yellow orange colour will be produced which will indicate the presence of flavones.

**c. Acid Test**

To the small quantity of test solution, few drops of concentrated sulphuric acid were added. A yellow orange colour will be obtained which will indicate the presence of flavonols.

**7. Test for proteins**

**a. Millon's Test**

A small quantity of aciduous – alcoholic extract of the powdered drug was heated with Millon's reagent. A white precipitate turned red on heating will indicate the presence of proteins.

**b. Biuret Test**

To one portion of aciduous – alcoholic extract of the powdered drug 1mL of 10% sodium hydroxide solution was added, followed by one drop of dilute copper sulphate solution. A violet colour will be obtained which indicates the presence of proteins.

**8. Test for alkaloids**

About 2g of the powdered material was mixed with 1g of calcium hydroxide and 5mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5mL of dilute hydrochloric acid was added followed by 2mL of each of the following reagents. If the coloured precipitates are formed for the following reagents will indicate the presence of alkaloids

a) Mayer's Reagent - Cream precipitate

b) Dragendorff's Reagent - Orange brown precipitate

c) Hager's Reagent - Yellow precipitate

d) Wagner's Reagent - Reddish brown precipitate

**9. (i) Test for anthraquinone glycosides**

**a. Borntrager's Test**

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. A pink colour will be observed in the ammoniacal layer which shows the presence of anthraquinone glycosides.

**b. Modified Borntrager's Test**

About 0.1g of the powdered drug was boiled for 2min with dilute hydrochloric acid and few drops of ferric chloride solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract. A pink colour will be observed in ammoniacal layer which shows the presence of anthraquinone glycosides.

**(ii) Test for cardiac glycosides (for deoxysugar)**

**Keller Kiliani Test**

About 1g of the powdered leaf was boiled with 10mL of 70% alcohol for 2min, cooled and filtered. To the filtrate 10mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid. To this 2 drops of ferric chloride solution was added. Then 3 mL of concentrated sulphuric acid was added to the sides of the test tube carefully and observed. A reddish brown layer will be observed which indicates the presence of deoxysugars of cardiac glycosides.

**(iii) Test for cyanogenetic glycosides**

A small quantity of the powder was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2h in a warm place. A brick red color produced on the paper will indicate the presence of cyanogenetic glycosides.

**10. Test for saponins**

**Foam test**

About 0.5g of the powdered drug was boiled gently for 2min with 20mL of water and filtered while hot and allowed to cool. 5mL of the filtrate was then diluted with water and shaken vigorously. Frothing if produced indicates the presence of saponins.

**11. Test for tannins**

A small quantity of the powdered drug was extracted with water. To the aqueous extract, few drops of ferric chloride solution was added. A bluish black colour if produced will indicate the presence of tannins.

The results obtained for the qualitative chemical tests for both leaf powder and extracts are presented in **Table 7 & 8.**

**Quantitative estimation of phyto-constituents**

The quantitative estimations are important since they help in finding the amount of secondary metabolites present in the crude extracts which can be expressed as equivalent to a standard drug. This helps us to find which secondary metabolites are responsible for the pharmacological activity

**Estimation of total Phenolic compounds [67]**

**Principle**

The total phenolic content of the 70% ethanolic extract of *Ipomoea pes-tigridis* was determined by Folin-Ciocalteu reagent method. The phenolic compounds are oxidised by the

Folin-Ciocalteu reagent. The Folin reagent is a mixture of phospo molybdate and phosphotungstate. The reagent is reduced, during oxidation of the phenolic substances into a mixture of blue molybdenum and tungsten oxides. So this method measures the amount of substance needed to inhibit the oxidation of the reagent. The blue colour produced has a maximum absorption at about 750-760nm. The absorption is proportional to the quantity of oxidised phenolic compounds.

**Reagents**

a) Folin Ciocalteu Reagent (1N)

   Commercially available Folin Ciocalteu Reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in an amber coloured bottle and stored in refrigerator at 4ºC.

b) Sodium carbonate solution (10%)

c) Standard gallic acid solution.

d) A stock solution of 70% ethanolic extract (1mg/mL)

**Procedure**

To the1mL of the stock solution of *Ipomoea pes-tigridis* in a test tube, 0.5mL of Folin Ciocalteu reagent (1N) was added and allowed to stand for 15min. Then 1mL of 10% sodium carbonate solution was added. Finally the mixture was mixed with distilled water and made upto10mL, allowed to stand for 30min at room temperature and total phenol was determined spectrophotometrically at 760nm. Gallic acid was used a reference compound. A calibration curve of concentration versus absorbance was generated for a standard drug namely gallic acid at different concentrations (2, 4, 6, 8, 10µg/mL). The reaction mixture without sample was used as blank. The total phenol content was calculated using regression analysis and was expressed in terms mg of gallic acid equivalent/g of extract (mg GAE/g) and the results obtained are presented in **Table 9.**

**Estimation of total Flavanoid content [68-70]**

**Principle**

The aluminium chloride colorimetric technique was used for estimation of total flavonoid estimation. The aluminium ions form stable complexes with C4 keto group and either to C3 or C5 hydroxyl groups of flavones and flavonols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavonoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids.

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

10% aluminum chloride

1M potassium acetate

**Procedure**

An aliquot quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg/mL. Further dilutions were made to get concentrations ranging from 20-100µg/mL. 1ml of the above standard solutions were taken in different volumetric flasks, 0.1mL of aluminum chloride solution, 0.1mL of potassium acetate solution and 2.8mL of ethanol were added and the final volume was then made up to 5mL with distilled water. After 20min the absorbance was measured at 415nm. A sample without aluminium chloride was used as a blank. From the absorbance obtained, a calibration curve was constructed by plotting concentration versus absorbance of quercetin (**Fig. 13).** 1mL of 70%ethanolic extract of sample at concentrations 100µg/mL and 200µg/mL were taken and the reaction was carried out as above and the absorbance was measured at 415nm after 20min and the readings are tabulated in **Table 10.**

The amount of flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg querecetin equivalents /g of extract. The results obtained are presented in **Table 10.**

## CHROMATOGRAPHY

Chromatography may be defined as the method of separating a multi component mixture into individual components through equiliburium distribution between two phases. The technique of chromatography was first invented by M.Tswett a botanist in 1906 in Warsaw. Tswett termed chroma and graphos means "colour" and "writing" respectively. The technique is mainly based on the differences in the rate at which the components of mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase) [71].

### The steps involved in the chromatographic methods

- Adsorption or retention of substance or substances on the stationary phase
- Separation of adsorbed substances by mobile phase
- Recovery of separated substances by continuous flow of mobile phase the method being called elution
- Quantitative and qualitative analysis

### Types of chromatographic methods

Chromatographic methods can be classified according to the nature of the stationary and mobile phases. If the stationary phase is a solid, the process is called as adsorption chromatography and if the stationary phase is a liquid, it is termed as partition chromatography. The various types of chromatography includes paper chromatography (PC), thin layer chromatography (TLC), column chromatography (CC), gas chromatography (GC),

high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC)

**Thin Layer Chromatography [72-75]**

Thin layer chromatography was accepted as a separation method of the analytical laboratory after suitable sorbents became available for self preparation of thin layer plates. This development took place at the beginning of the 1960s. Traditionally analytical TLC has found application in the detection and monitoring of compounds through a separation process. In case of known natural products or other compounds (pharmaceuticals) qualitative and quantitative information can be gathered concerning the presence or absence of the metabolite or breakdown product. The history of TLC dates back to 1938 when Izmailov and Shraiber separated plant extracts using 2mm thick and firm layer of alumina set on glass plate. In 1944, Consden and Martin used filter papers for separating amino acids. In 1950, Kirchner identified terpenes on filter paper and later glass fibre coated with alumina. Only in 1958, Stahl develop standard equipment for analysing by TLC.

**TLC studies of 70% ethanolic extract of *Ipomoea pes-tigridis***

**Principle**

The principle of separation is adsorption. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase will travel slower and the components with less affinity towards the stationary phase travel faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

**Preparation of TLC Plates**

A slurry of the adsorbent (silica gel G) was prepared in water (1 : 2). Dry, clean glass plates (20cm x 5cm) were laid in a row as a template, the suspension was poured into Stahl

TLC spreader, which was adjusted to 0.25mm thickness and coated in a single passage of the spreader over them. These plates were left on the template for air drying until the transparency of the layer disappeared and dried at 110$^{o}$C for 30min and kept in a dessicator. The plates were used when required. Aluminium plates coated with silica gel G $F_{254}$ (Merck) were also used.

**Application of Extract**

The 70% Ethanolic extract of *Ipomoea pes-tigridis* was suitably diluted using ethanol as solvent. This solution was taken in a capillary tube and it was spotted on TLC plate 2cm above the bottom end.

**Development of chromatogram**.

The TLC plates were developed in a chromatographic tank by using our different mobile phases viz., solvent system I, solvent system II, solvent system III and solvent system IV. The plates were allowed to develop 3/4 of the length and then removed. The solvent front was immediately marked and the plates were allowed to dry and then it was examined by visually, under UV light at 254 and 366nm or various chemical reagents (Folin Ciocalteu reagent (2N). The results are presented in **Table 11 & 12.**

Stationary phase - Silicagel G

Mobile phase - Toluene : Ethylacetate : Formic acid (12: 6: 0.05)

- Toluene : Acetone : Formic acid (30 : 10 :1)
- Toluene : Ethylacetate (7.5: 2.5)
- Chloroform : Ethylacetate (60:40)

Detecting agent - Visual & UV light, Folin ciocalteu reagent (2N).

The $R_f$ value of the spots obtained were calculated using the formula,

$$\mathbf{R_f\ value} = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent}}$$

The TLC plates were examined under UV light and ordinary light. The spots seen in the ordinary light are shown in **Fig. 14** and the $R_f$ value of the spots are presented in **Table 11.**

**High Performance Thin Layer Chromatography (HPTLC) [74, 75]**

HPTLC method is a most improved advancement of Thin Layer chromatography (TLC). It has added advantages like better resolution, faster development of spots and also easy detection and quantification of separated compounds. HPTLC is useful for identification of plants and their extracts because each plant species produces a distinct chromatogram, with unique marker compounds which can be used for plant identification. It is used as a quality control tool since comparison of chromatograms of different lots can demonstrate the similarities and differences between the test samples and the standard chemical markers. HPTLC is a reliable method for quantitation of nanogram level even when present in complex formulation. HPTLC finger print analysis is used for rapid identity check, for monitoring purity of drugs, for detection of adulterants, for determining whether a material is derived from a defined botanical species and also to known whether the constituents are clearly characterized.

**Development of HPTLC fingerprint**

**Instrument**

CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was winCATS 1.4.3

**Sample**

The 70% ethanolic extract of *Ipomoea pes-tigridis* was dissolved in ethanol to get a concentration of 10mg/mL and 2µL of this solution applied as 8mm band and was used for taking HPTLC fingerprint.

**Stationary Phase**

Aluminium sheets pre-coated with silica gel Merck G $F_{254}$, 0.2mm layer thickness were used as the stationary phase.

**Mobile phase**

Toluene: Ethyl acetate : 100% formic acid (12 : 6 : 0.1) was used as the mobile phase for developing the chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber.

**Detection wavelength**

The developed plates were examined at wavelength 254 and 366nm.

## RESULTS AND DISSCUSSION

### Preliminary phytochemical screening

The results obtained for the preliminary phytochemical screening of the leaf powder are depicted in **Table 7** and it can be seen that the leaf powder of *Ipomoea pes-tigridis* showed the presence of carbohydrates, proteins, terpenoids, steroids, phenolic compounds, flavonoids and tannins.

**Table 7: Preliminary phytochemical screening of the leaf powder of *Ipomoea pes-tigridis* Linn**

| S.No | TEST | RESULTS |
|---|---|---|
| **1.** | **TEST FOR STEROLS** | |
| | a. Salkowski's test | + |
| | b. Libermann- Burchard's test | + |
| **2.** | **TEST FOR CARBOHYDRATES** | |
| | a. Molisch's test | + |
| | b. Fehling's test | + |
| | c. Benedict's test | + |
| **3.** | **TEST FOR PROTEINS** | |
| | a. Millon's test | + |
| | b. Biuret test | + |
| **4.** | **TEST FOR ALKALOIDS** | |
| | a. Mayer's reagent | - |
| | b. Dragendroff's reagent | - |
| | c. Hager's reagent | - |
| | d. Wagner's reagent | - |
| **5.** | **TEST FOR GLYCOSIDES** | |
| | a. Anthraquinone glycosides | |
| | i) Borntrager's test | - |
| | ii) Modified Borntrager's test | - |
| | b. Cardiac glycosides | |
| | i) Keller Killiani test | - |
| | c. Cyanogenetic glycosides | - |
| **6.** | **TEST FOR SAPONINS** | |
| | Foam test | - |
| **7.** | **TEST FOR TANNINS** | |
| | $FeCl_3$ test | + |
| **8.** | **TEST FOR FLAVONOIDS** | |
| | a. Shinoda test | + |
| | b. Alkali test | + |
| | c. Acid test | + |
| **9.** | **TEST FOR TERPENOIDS** | + |
| **10.** | **TEST FOR VOLATILE OILS** | - |

**(+) indicates positive reaction (-) indicates negative reaction**

The preliminary phytochemical screening for the various extracts of the leaf powder of *Ipomoea pes-tigridis* are presented in **Table 8.**

**Table 8: Preliminary phytochemical screening of the various extracts of leaf powder of *Ipomoea pes-tigridis***

| Tests | Pet. ether Extract | Hexane Extract | Chloro form Extract | Acetone Extract | Ethyl acetate extract | Methanol Extract | Ethanol Extract | Ethanol extract (70%) | Aqueous extract |
|---|---|---|---|---|---|---|---|---|---|
| **I. STEROLS** | | | | | | | | | |
| Salkowski's test | + | - | - | - | - | - | - | - | - |
| Libermann-Burchard's test | + | - | - | - | - | - | - | - | - |
| **II. Test for Carbohydrates** | | | | | | | | | |
| Molisch's test | - | - | - | - | - | + | + | + | + |
| Fehling's test | - | - | - | - | - | + | + | + | + |
| Benedict's test | - | - | - | - | - | + | + | + | + |
| **III.Test for Proteins** | | | | | | | | | |
| Millon's test | - | - | - | - | - | + | + | + | + |
| Biuret test | - | | - | - | - | + | + | + | + |
| **IV.Test for Alkaloids** | | | | | | | | | |
| Mayer's reagent | - | - | - | - | - | - | - | - | - |
| Dragendroff's reagent | - | - | - | - | - | - | - | - | - |
| Hager's reagent | - | - | - | - | - | - | - | - | - |
| Wagner's reagent | - | - | - | - | - | - | - | - | - |
| **V.Test for Glycosides** | | | | | | | | | |
| Anthraquinone glycosides | - | | - | - | - | - | - | - | - |
| i) Borntrager's test | - | | - | - | - | - | - | - | - |
| ii) Modified Borntrager's test | - | | - | - | - | - | - | - | - |
| Cardiac glycosides | | | | | | | | | |
| i) Keller Killiani test | - | - | - | - | - | - | - | - | - |
| Cyanogenetic glycosides | - | - | - | - | - | - | - | - | - |
| **VI.Test for Saponins** | - | - | - | - | - | - | - | - | - |
| **VII.Test for Tannins** | | | | | | | | | |
| $FeCl_3$ test | - | - | - | - | + | + | + | + | + |
| Lead acetate Test | - | - | - | - | + | + | + | + | + |
| **VIII.Test for Flavonoids** | | | | | | | | | |
| Shinoda test | - | - | - | - | - | + | + | + | + |
| Alkali test | - | - | - | - | - | + | + | + | + |
| Acid test | - | - | - | - | - | + | + | + | + |
| **IX.Test for Terpenoid** | - | - | + | - | - | + | + | + | - |

From the **Table 8,** it can be observed that the methanol ethanol and 70% ethanolic extracts showed the presence of carbohydrates, proteins, flavonoids and terpenoids while the

petroleum ether extract showed the presence of sterols. The aqueous extract showed the presence of carbohydrates, proteins and flavonoids.

## QUANTITATIVE ANALYSIS

### Estimation of total phenolic content

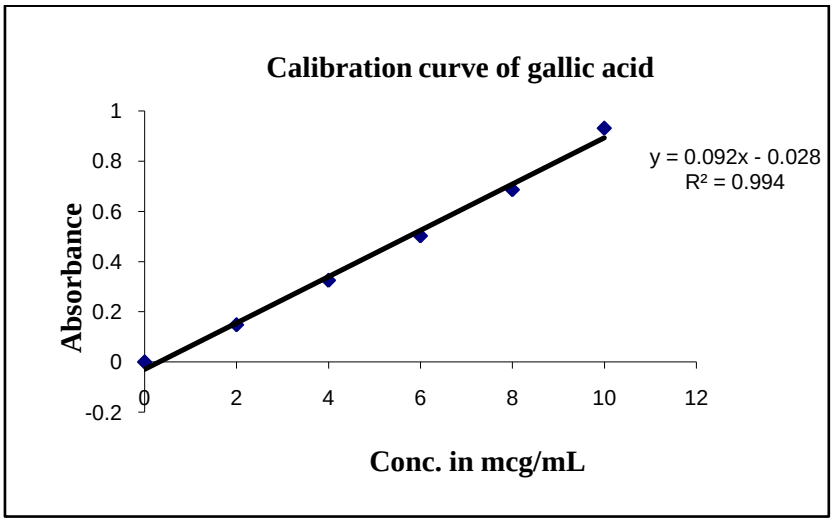
The results obtained for the total phenolic content in 70% ethanolic extract of *Ipomoea pes- tigridis* is presented in **Table 9** and **Fig. 12.** The linear regression equation was found to be y = 0.116x - 0.004 while the correlation coefficient was found to be 0.9998 for gallic acid. The amount of phenolic content present in the ethanolic extract in terms of mg GAE/g of extract was found to be 51.41±5.37 by using the above regression equation.

**Table 9: Total phenolic content in hydro alcoholic extract of *Ipomoea pes- tigridis* in terms of gallicacid equivalents**

| S. No. | Conc. of gallic acid in µg/mL | Absorbance at 760nm | Conc. of 70% ethanolic extract in µg/mL | Absorbance at 760nm* | Amount of total phenolic content in terms mgGAE/g of extract* |
|---|---|---|---|---|---|
| 1 | 2 | 0.229 ± 0.010 | 50 | 0.257±0.001 | 44.833±0.44 |
| 2 | 4 | 0.452 ± 0.006 | 100 | 0.672±0.004 | 58±0.471 |
| 3 | 6 | 0.695 ± 0.005 | | Average | **51.41±5.37** |
| 4 | 8 | 0.918 ± 0.028 | | | |
| 5 | 10 | 1.162 ± 0.031 | | | |

***Mean of three readings± SEM**

**Fig. 12: Calibration curve of gallic acid**




**Estimation of total flavonoid content**

The results for the total flavonoid content in 70% extract of *Ipomoea pes-tigridis* are presented in **Table 10** and **Fig. 13.**

**Table 10: Total flavanoid content per gram of ethanolic (70%) extract in terms of quercetin by aluminium chloride method**

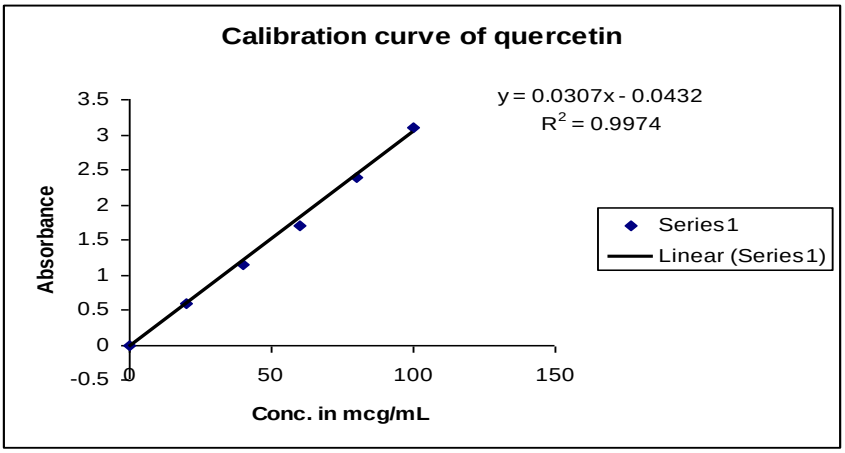
| S. No. | Conc. of quercetin in µg/mL | Absorbance at 415nm | Conc. of ethanolic extract in µg/mL | Absorbance at 415nm | Amt of total flavonoid content in terms mg quercetin equivalent/ g of extract |
|---|---|---|---|---|---|
| 1 | 20 | 0.589 ± 0.01 | 100 | 0.039± 0.0003 | 26.80 ± 0.066 |
| 2 | 40 | 1.151 ± 0.04 | 200 | 0.082±0.0034 | 20.39 ± 0.060 |
| 3 | 60 | 1.710 ± 0.09 | | | |
| 4 | 80 | 2.390 ± 0.03 | | | |
| 5 | 100 | 3.112 ± 0.03 | | | |
| | | | | Average | **23.59** |

***Mean of three readings ± SEM**

The linear regression equation was found to be 0.0307x - 0.0432 while the correlation coefficient was found to be 0.998 for quercetin. The amount of flavonoid content in terms of

mg quercetin equivalent/g of 70% ethanolic extract of *Ipomoea pes-tigridis* was found to be 23.59±0.066 by using the above linear equation.

**Fig. 13: Calibration curve of quercetin**




## CHROMOROGRAPHY

### Thin layer chromatography

The results obtained for evaluation of phyto constituents of 70% ethanolic extract by TLC with various solvent systems are presented in **Table 11** and the photographs of the TLC plates are presented in **Fig. 14.** From the **Table 11,** it can be observed that the 70% ethanolic extract of *I. pes-gridis* when developed with chloroform & ethyl acetate (60:40) as mobile phase showed 4 spots under UV light and under ordinary light. The same when developed using toluene and ethyl acetate (7.5:2.5) and toluene, acetone and formic acid (30:10:0.1) as mobile phases, showed 4 spots under UV light and 7 spots under ordinary light. The extract when developed with toluene, ethyl acetate and formic acid (12:6:0.1) as mobile phase showed 4 spots under UV light and 8 spots at ordinary light. All the plates developed showed a common spot at a $R_f$ value of 0.7 which was yellow in all the systems developed. This spot may be a flavonoid compound [72]. Further evaluation of the same is necessary

**Table 11: Phytochemical evaluation of 70% ethanolic extract of *Ipomoea pes- tigridis* by TLC studies**

| Solvent System | UV | | | DAY LIGHT | | |
|---|---|---|---|---|---|---|
| | **No. of spots** | **$R_f$** | **Colour** | **No. of spots** | **$R_f$** | **Colour** |
| Chloroform: Ethyl acetate 60:40 | **4** | | | **4** | | |
| | 1 | 0.79 | Orange Fluorescence | 1 | 0.79 | Yellow |
| | 2 | 0.61 | Orange | 2 | 0.61 | Yellow |
| | 3 | 0.38 | Yellowish Orange | 3 | 0.38 | Green |
| | 4 | 0.12 | Pale Orange | 4 | 0.12 | Pale Green |
| Toluene: Ethyl acetate 7.5 : 2.5 | **4** | | | **7** | | |
| | 1 | 0.72 | Orange fluorescence | 1 | 0.72 | Dark Yellow |
| | 2 | 0.65 | Orange | 2 | 0.65 | Yellow |
| | 3 | 0.57 | Pale orange | 3 | 0.57 | Grey |
| | 4 | 0.12 | Pale orange | 4 | 0.51 | Green |
| | | | | 5 | 0.46 | Light Green |
| | | | | 6 | 0.33 | Yellowish green |
| | | | | 7 | 0.22 | Pale green |
| Toluene: ethylacetate : formic acid 12:6:0.1 | **4** | | | **8** | | |
| | 1 | 0.73 | Dark orange | 1 | 0.73 | Yellow |
| | 2 | 0.65 | Orange | 2 | 0.65 | Dark yellow |
| | 3 | 0.53 | Pale orange | 3 | 0.53 | Pale yellow |
| | 4 | 0.47 | Orange | 4 | 0.47 | Grey |
| | | | | 5 | 0.38 | Green |
| | | | | 6 | 0.25 | Pale yellow |
| | | | | 7 | 0.2 | Pale green |
| | | | | 8 | 0.15 | Green |
| Toluene :acetone : Formic acid 30:10:0.1 | **4** | | | **7** | | |
| | 1 | 0.82 | Dark orange | 1 | 0.82 | Yellow |
| | 2 | 0.72 | Orange | 2 | 0.72 | Dark yellow |
| | 3 | 0.64 | Orange | 3 | 0.64 | Pale yellow |
| | 4 | 0.54 | Pale orange | 4 | 0.54 | Pale Green |
| | | | | 5 | 0.45 | Green |
| | | | | 6 | 0.41 | Green |
| | | | | 7 | 0.33 | Pale green |

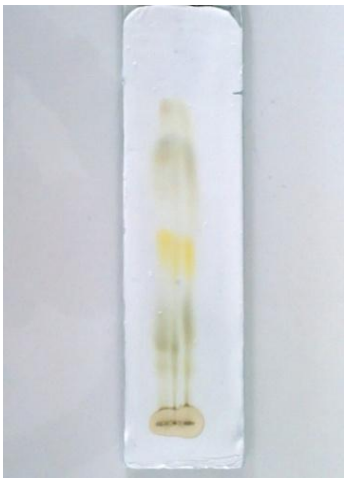
The TLC plates when examined after using different detecting agents (**Table 12 & Fig. 14)** showed the presence of different active principles in the 70% ethanolic extract of *Ipomoea pes-tigridis.* The Folin Ciocalteu's reagent sprayed plate showed 4 spots with $R_f$ values, 0.07, 0.54, 0.76 and 0.90. Out of which the spot at $R_f$ of 0.07 (blue colour) may be due to the presence of phenolic compounds [73, 75].

**Table 12: TLC finger profile of the 70% ethanolic extract of *Ipomoea pes-tigridis***

| Solvent system | Detecting agent | No of spots | Color of spots | $R_f$ values |
|---|---|---|---|---|
| Toluene:ethylacetate:formic acid (12: 6: 0.1) | Folin Ciocalteu's reagent | **4** | | |
| | | 1 | Dark blue | 0.07 |
| | | 2 | Blue | 0.54 |
| | | 3 | Blue | 0.76 |
| | | 4 | Blue | 0.90 |
| | Under UV light | **6** | **at 366nm** | |
| | | 1 | Fluorescence | 0.07 |
| | | 2 | Pinkish red | 0.52 |
| | | 3 | Pinkish red | 0.56 |
| | | 4 | Pinkish red | 0.66 |
| | | 5 | Pinkish red | 0.78 |
| | | 6 | Pinkish red | 0.83 |

**Fig. 14.1: TLC analysis 70% ethanolic extract of *Ipomoea pes-tigridis* in day light**

**Toluene:ethyl acetate: formic acid (12:6:0.1)**



**Toluene: acetone: formic acid (30:10:5)**

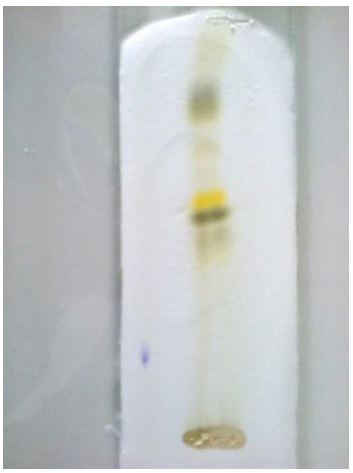


**Fig. 14.2: TLC analysis of 70% ethanolic extract of *Ipomoea pes-tigridis* in day light**

**Chloroform : Ethyl acetate (60:40)**

**Toluene : Ethyl acetate (7.5: 2.5)**

From the **Table 8** and **Fig. 14** the visualization of TLC plate of ethanolic (70%) extract of *Ipomoea pes-tigridis* at daylight showed yellow color spot in above four solvent system at a $R_f$ value 0.7 which may be due to presence of flavonoid compound [73].

**HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY**

The TLC visualization, 3D display of the finger print profile and peak display at 254nm and 366nm are presented in **Table 13** and **Fig. 15.** The photo of the TLC plate at 254nm shows the presence of 8 spots while at 366nm shows the presence 12 spots. The 3D display of the fingerprint profile and the peak display of 70% ethanolic extract of *Ipomoea pes-tigridis* at 254nm and 366nm is presented in **Fig. 16**. The display at 254nm shows the presence of 8 peaks while at 366nm shows the presence of 12 peaks. The $R_f$ values of the peaks along with the area under the curve for each peak at 254 and 366 nm are tabulated in **Table 13.**

**Fig. 15: Visualization of 70% ethanolic extract of *Ipomoea pes-tigridis* at 254nm and 366nm**

**254nm** **366nm**

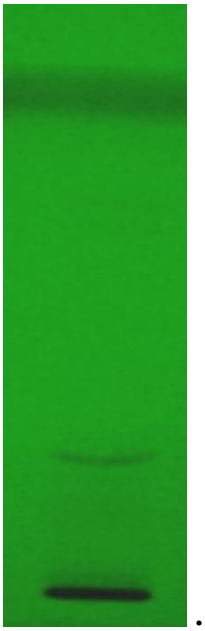
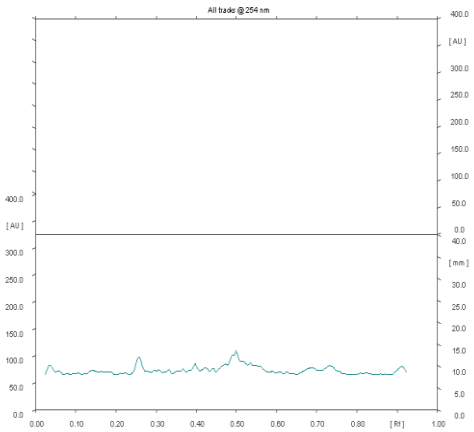


**Fig. 16: 3D Display of the fingerprint profile at 254nm and 366nm**

**@ 254nm** **@ 366nm**



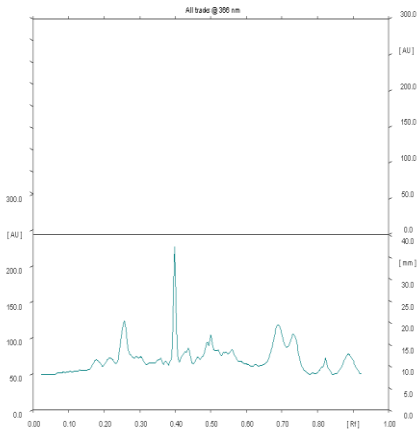



**Fig.17: Peak display of hydroalcoholic extract of *Ipomoea pes-tigridis* at 254nm and 366nm**

**@ 254nm** **@ 366nm**

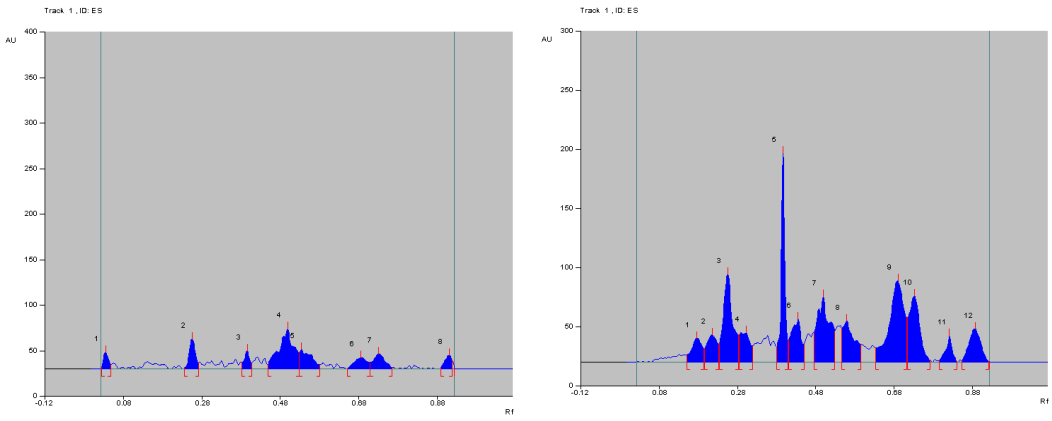


**Table 13: $R_f$ values and area under the curve for each peak at 254 nm and 366nm**

| At 254nm | | | At 366nm | | |
|---|---|---|---|---|---|
| **Peak** | **$R_f$ value** | **Area (AU)** | **Peak** | **$R_f$ value** | **Area (AU)** |
| 1 | 0.04 | 185.8 | 1 | 0.18 | 417.9 |
| 2 | 0.26 | 402.4 | 2 | 0.22 | 463.9 |
| 3 | 0.40 | 207.4 | 3 | 0.26 | 1377.6 |
| 4 | 0.50 | 1260.4 | 4 | 0.30 | 513.0 |
| 5 | 0.53 | 484.8 | 5 | 0.40 | 1304.3 |
| 6 | 0.69 | 310.7 | 6 | 0.44 | 704.3 |
| 7 | 0.73 | 371.2 | 7 | 0.50 | 1373.0 |
| 8 | 0.91 | 199.4 | 8 | 0.56 | 795.3 |
| | | | 9 | 0.69 | 1997.1 |
| | | | 10 | 0.73 | 1192.8 |
| | | | 11 | 0.82 | 274.4 |
| | | | 12 | 0.89 | 658.7 |

The HPTLC fingerprint profiles helps in identification of the plant, crude material and powdered drug and also in impurity profiling of the drug since no two fingerprint profile of the plant extracts will be same and hence useful in identification of the particular material.

# CHAPTER - 7

## PHARMACOLOGICAL EVALUATION

Pharmacological screening procedures are important and necessary in order to estimate the therapeutic potential of a drug. The classical method of pharmacological screening involves sequential testing of any new chemical compound or extract from herbal sources by i*n vitro* and *in viv*o experiments. Most of the extracts or drugs used in therapy have been found and evaluated with these methods.

The 70% ethanolic extract of the leaves of *Ipomoea pes-tigridis* was screened for various pharmacological activities like

- ❖ Antioxidant activity
- ❖ Anti inflammatory activity by membrane stabilization
- ❖ Anti arthritic activity by protein denaturation
- ❖ Cytotoxic studies on liver HEP G2 Cell lines
- ❖ Antibacterial activity

**Antioxidant Activity**

The antioxidants are micro nutrients that are able to neutralize free radicals or their actions. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to start a chain reaction. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols [76].

**Natural Antioxidants**

Natural antioxidants are called as body´s detoxifiers. They are chemically "free radicals" that do not give away electrons because they are stable in their own way. They convert the toxins of the body into harmless waste products that are naturally excreted from the body [77]. The antioxidant rich foods are natural cleansers of the body and act as antiaging agents. They absorb bad cholesterol and substantially reduce the risk of heart diseases. Red kidney beans, cloves, bell pepper, tea, vitamin C and vitamin E containing foods are some of the best examples for natural antioxidants.

Vitamin E, ascorbic acid and phenolic constituents possess the ability to decrease the oxidative damage caused in many diseases like diabetes, cancer and ageing. Antioxidant compounds have many functions like free radical scavenging, quenching of singlet oxygen formation and reducing agents [78- 80].

Synthetic antioxidants like butylated hydroxy toluene and butylated hydroxyl anisole are carcinogenic and hence they are find restricted use in foods. Therefore the seach for natural antioxidants has increased in recent years. A number of crude extracts have been reported to possess antioxidant properties.

The assessment of antioxidant property can be carried out by both *in vitro* and *in vivo* models. Some of the *in vitro* models used for the evaluation of antioxidant activity are listed below [81]

- ❖ DPPH method
- ❖ Hydrogen peroxide method
- ❖ Superoxide radical scavenging activity
- ❖ Hydroxyl radical scavenging activity

- ❖ Nitric oxide radical inhibition assay
- ❖ Reducing power method
- ❖ Phosphomolybdenum method
- ❖ Peroxy nitrile radical scavenging activity
- ❖ Xanthine oxidase method
- ❖ Ferric reducing ability of Plasma
- ❖ Thiobarbituric acid assay
- ❖ ABTS radical scavenging method etc.

**Method 1: Diphenyl picryl hydrazyl (DPPH) method [ 82, 83, 84]**

**Principle:**

DPPH scavenging activity or hydrogen donating capacity was quantified in presence of stable DPPH on the basis of Blois method [84]. Its reaction with antioxidants can be followed by the loss of absorbance at 517nm. It is widely accepted that DPPH can accept an electron or hydrogen radical and become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH (purple colour solution) shows a strong absorption at 517nm. DPPH radicals react with suitable reducing agents where the pairing of electrons takes place and the solution loses colour stochiometrically with the number of electrons taken up.

$$\mathbf{DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}}$$

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

0.1mM diphenyl picryl hydrazyl in ethanol

**Procedure**

To the 1mL of test samples of different concentrations, 4mL of DPPH was added. A control without test compound was prepared in an identical manner. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance of the test mixtures was read at 517nm against a blank solution of ethanol instead of DPPH. Ascorbic acid was used as a standard. The percentage inhibition was calculated and expressed as percentage scavenging of DPPH radical. The percentage scavenging was calculated using the formula

**%inhibition = [(Control-Test)/Control] x 100**

A plot of concentration versus percentage inhibition was plotted **(Fig. 18)** and the linear regression equation calculated. The concentration of the sample required for 50% reduction in absorbance ($IC_{50}$) was calculated using linear regression analysis. The results obtained are presented in **Table 14.**

**Nitric oxide scavenging activity assay [85, 86]**

**Principle**

Nitric oxide scavenging activity was determined according to the method reported by Green *et al.,* 1982 [85]. Nitric oxide is spontaneously generated when sodium nitropruside in aqueous solution is maintained at physiological pH. The nitric oxide formed interact with oxygen to produce nitrite ions. The nitrite ions produced diazotizes sulphanilamide to a diazonium salt which reacts with NN naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 546nm.

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

10mM sodium nitroprusside

Phosphate buffered saline pH 7.4

2% sulphanilamide in ortho phosphoric acid

0.1% naphthyl ethylene diamine dihydrochloride

**Procedure**

To 1mL of sodium nitroprusside in 2.5mL phosphate buffered saline (pH 7.4), 1mL of extracts at various concentrations were added and the mixtures were incubated at 25$^{o}$C for 30min. To 1.5mL of the incubated mixture, 1mL of sulphanilamide in phosphoric acid and 0.5mL of naphthyl ethylene diamine dihydrochloride were added. The absorbance was measured at 546nm using reagent as blank. Ascorbic acid was used as a standard. The percentage inhibition of nitric oxide radical generated was calculated using the following formula:

**% inhibition = [(Control-Test)/Control] x 100.**

A graph of concentration versus percentage inhibition was plotted and the regression equation was calculated **(Fig.n19).** The **$IC_{50}$** for the 70% ethanolic extract was calculated using linear regression equation. The results obtained are presented in **Table 15.**

**Method 3: Reducing power assay [87, 88]**

**Principle**

The assay is based on the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent reaction with ferric chloride to form a blue colour which has a maximum absorption at 700nm. An increase in absorbance of the reaction mixture as concentration increases shows an increased antioxidant activity.

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

1% potassium ferricyanide

10% trichloro acetic acid

0.2M, pH 6.6 phosphate buffer

0.1% ferric chloride

**Procedure**

To 1mL of different concentrations of 70% ethanolic extract solution, 2.5mL phosphate buffer and 2.5mL potassium ferricyanide were added and incubated at 50$^{o}$C for 20min. The mixture was then removed and 2.5of trichloro acetic acid was added and centrifuged at 3000rpm for 10min. Finally, 1.5mL of the supernatant solution was mixed with 1.5mL of distilled water and 0.5mL ferric chloride and the absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of three parallel experiments was expressed as mean ± standard error of the mean. A graph was plotted using concentration versus absorbance **(Fig. 20 & 21).** The antioxidant activity was expressed as equivalents of Vitamin C (µg/mL). The results obtained are presented in **Table 16.**

**Method 4: Determination of scavenging activity against hydrogen peroxide [89, 90]**

**Principle:**

The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Ruch *et al* [90]. The principle is based the capacity of the extracts to decompose the hydrogen peroxide to water.

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

6% hydrogen peroxide diluted with water in the ratio of 1:10

0.1M phosphate buffer (pH 7.4)

**Procedure**

To 1mL of various concentrations of plant extract, 3.8mL of 0.1M phosphate buffer solution (pH 7.4) was added and then mixed with 0.2mL of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min using a reaction mixture without sample as blank. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control} \times 100.$$

A graph of concentration versus percentage inhibition was plotted **(Fig. 22)** and the linear regression equation was calculated. The $IC_{50}$ value was calculated using linear regression analysis. The results obtained are presented in **Table 17.**

**Anti –Inflammatory activity [92-100]**

Inflammation is a basic reaction of living tissue to injury and it comprises of a series of changes of the terminal vascular blood and connective tissues. The inflammatory response occurs in three distinct phases. They are mediated by different mechanisms [91].

a. Acute transient phase : It is characterized by local vasodilatation and increased capillary permeability.

b. Sub acute phase : It is characterized by infilteration of leucocytes and phagocytes.

c. Chronic proliferative phase : It is characterized by occurrence of tissue degeneration and fibrosis.

In the development of numerous methods of assay for detecting anti inflammatory substances, various *in vitro* and *in vivo* models have been proposed as being able to detect anti-inflammatory effect with related to beneficial effect in rheumatoid activity.

***In vitro* membrane stabilisation study**

**Principle**

The anti-inflammatory activity by HRBC method is simple, reliable, less toxic and less time consuming method [92, 93]. Since HRBC membrane is similar to the lysosomal membrane which influence in the process of inflammation. The lysosomal enzymes released during inflammation produces a variety of disorders. The extracellular activity of these enzymes are said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane.

The method of Sadique *et al*., 1989 [94] and modified by Oyedapo and Famurewa 1995 [95] and Oyedapo *et al.,* 2002 [99] was employed. RBCs are subjected to heat and to treatment with hyposaline they undergo lysis and release haemoglobin. The released haemoglobin has a maximum absorbance at about 560nm. The capacity of the extract to reduce hyposaline and heat induced lysis is the basis for the assay [96-98].

**Instrument**

Shimadzu UV Visible spectrophotometer, Model 1800

**Reagents**

0.2M sodium phosphate buffer (pH 7.4)

0.36% w/v hyposaline

10%v/v HRBC suspension in isosaline

**Preparation of HRBC suspension in isosaline**

The human erythrocytes suspension was used for the *in vitro* membrane stabilization assay. The blood was collected from healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever solution (2% dextrose, 8.0% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) and centrifuged at 3000rpm. The packed cells were washed with isosaline and a 10% v/v erythrocyte suspension in isosaline was prepared.

**Procedure**

The 70% ethanolic extract of *Ipomoea pes-tigridis* was dissolved in ethanol to get a stock solution of 2mg/mL. From this, varying volumes of the extract (0.2 to 1mL) was added to 2mL of hyposaline and 1mL of phosphate buffer and 0.5mL HRBC suspension in isosaline, then the final volume were made up with isosaline up to 4.5mL. The control was prepared as mentioned above except the drug was omitted, while drug control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56ºC for 30min on a water bath, then the tubes were cooled under running water. Then the absorbance of the released haemoglobin was measured at 560nm. Diclofenac sodium was used as a reference standard. The percentage membrane stabilisation activity of the compounds were determined by the formula

$$\% \text{ Membrane stabilization} = [A_{control} - (A_{test} - A_{product\ control})] / A_{control} \times 100$$

$A_{control}$ - Absorbance in control

$A_{test}$ - Absorbance in test

$A_{product\ control}$ - Absorbance in product control

The results obtained for in vitro membrane stabilization effect are presented in **Table 18** and a graph was plotted using concentration versus percentage membrane stabilization **(Fig. 23).**

**Anti - Arthritic activity**

Arthritis is a complex and a widespread disease. The most common forms are osteoarthritis and rheumatoid arthritis. In osteoarthritis, the cartilage between two bones begins to wear down causing stress between two bones. This stress produces inflammation and occasionally bone spurs. Rheumatoid arthritis is an auto immune disease where the immune system actually begins attacking the cartilage and synovial linings in the joins between bones [105].

***In vitro* protein denaturation method [106-111]**

Rheumatoid arthritis is an autoimmune disorder. One of the causes for the disease is due to the denaturation of the proteins. Antiarthritic activity of the 70% ethanolic extract of *Ipomoea pes-tigridis* was assessed by *in vitro* protein denaturation method.

**Instrument**

UV- Visible spectrophotometer Model 1800

**Reagents**

Bovine serum albumin (5% w/v aqueous solution)

Phosphate buffer (pH 6.3)

**Procedure**

**Preparation of test solutions**

1. **Test solution:** The test solution consists of 0.45ml bovine serum albumin (5% w/v aqueous solution) and 0.05ml of extract (various concentrations of 70% ethanolic extract in µg/mL).

2. **Test control solution:** The test control solution consists of 0.45ml bovine serum albumin and 0.05ml distilled water.
3. **Product control:** The product control consists of 0.45ml distilled water and 0.05ml of extract (various concentrations of 70% ethanolic extract in µg/mL).
4. **Standard solution:** The standard solution consists of 0.45ml of bovine serum albumin and 0.05mL of diclofenac sodium solution

All the above test samples were adjusted to a pH 6.3 using a small amount of 1N hydrochloric acid. They were then incubated at 37°C for 20min and heated at 57° C for 3min and allowed to cool. 2.5ml of phosphate buffer (pH 6.3) was added to all the above solutions. The absorbance was measured using UV spectrophotometer at 416nm using phosphate buffer (pH 6.3) as blank. The percentage inhibition of protein denaturation was calculated using the formula.

$$\text{Percentage inhibition} = 100 - \left\{ \frac{(\text{OD of test solution} - \text{OD of product control})}{\text{OD of test control}} \times 100 \right\}$$

The control represents 100% protein denaturation. The results obtained for 70% ethanolic extract of *Ipomoea pes-tigridis* and the standard drug diclofenac sodium (250µg/ml) are presented in **Table 19.** A graph was plotted using concentration against percentage inhibition of protein denaturation for both the extract and diclofenac sodium (**Fig. 24).**

**Cytotoxicity studies**

An uncontrolled multiplication and spread within the body of abnormal form of body's own cells is called as "cancer". Combating cancer is of paramount importance today. An alternative solution to Western medicine which is embodied with severe side effects is the use of herbal preparations to arrest the insidious nature of the disease . Prior to 1983 in

United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural anti oxidants [114].

Free radical damage may lead to cancer. Antioxidants interact with radicals and may prevent some of the damage by free radicals. Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may show or possibly prevent the development of cancer [115].

**Hepatocellular carcinoma (Liver cancer) [116, 117]**

Hepatocellular carcinoma is cancer of the liver. This type of cancer occurs more often in men than in women. It is usually seen in people aged 50 or above. However, the age varies in different parts of the world. The disease is more common in parts of Africa and Asia than in North or South America and Europe. Hepatocellular carcinoma is not the same as metastatic liver cancer, which starts in another organ (such as the breast or colon) and spreads to the liver. In most cases, the cause of liver cancer is usually scarring of the liver (cirrhosis).

Cirrhosis may be caused by

- Alcohol abuse (the most common cause in the United States)
- Autoimmune diseases of the liver
- Hepatitis B or C virus infection
- Inflammation of the liver that is long-term (chronic)
- Iron overload in the body (hemochromatosis)

Patients with hepatitis B or C are at risk for liver cancer, even if they have not developed cirrhosis.

**Symptoms**

- Abdominal pain or tenderness, especially in the upper-right part

- Easy bruising or bleeding
- Enlarged abdomen
- Yellow skin or eyes (jaundice)

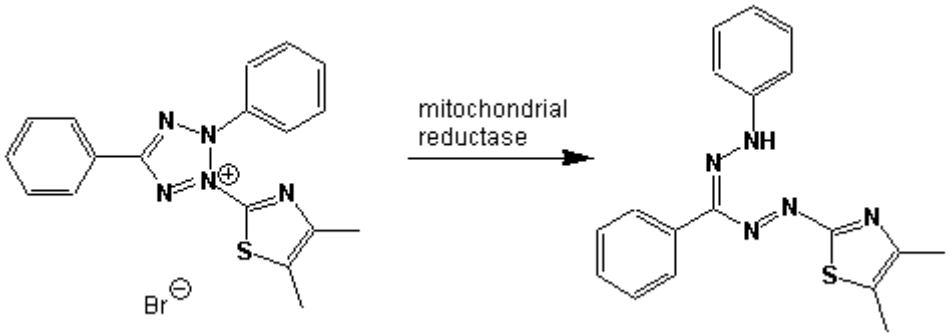
## *In Vitro* Anti Cancer Activity [118, 119]

HepG2 cells are a suitable *in vitro* model system for the study of polarized human hepatocytes. The human liver cancer cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at $37^0$C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

### Microculture tetrazolium (MTT) assay

### Principle

The method is based on the capacity of Mitochondria succinate-dehydrogenase enzymes in living cells which convert the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble purple coloured formazan product which is measured spectrophotometrically [117 ] Therefore, the amount of formazan produced is directly proportional to the number of viable cells.




**MTT** **FORMAZAN CRYSTALS**

**Methodology**

**Cell treatment procedure**

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of $1x10^5$ cells/ml. 100µL/well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at $37^0$C, 5% $CO_2$, 95% air and 100% relative humidity. After 24h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. 100µL/well of each concentration was added to plates to obtain final concentrations of 500, 250, 125, 62.5 and 31.25µg/ml. The final volume in each well was 200µL and the plates were incubated at $37^0$C, 5% $CO_2$, 95% air and 100% relative humidity for 48h. The medium without samples served as control. A triplicate was maintained for all concentrations.

. After 48h of incubation, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at $37^{\circ}$ C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µL of DMSO and then the absorbance was measured at 570nm using micro plate reader. The % cell inhibition was determined using the following formula

**% cell Inhibition = 100- Abs (sample)/Abs (control) x100.**

Nonlinear regression graph was plotted between % cell inhibition and $Log_{10}$ concentration and $IC_{50}$ was determined using Graph Pad Prism software. The results obtained are presented in **Table 20** and **Figs. 25 & 26.**

**Antibacterial Activity**

An antibacterial is a compound or substance that kills or slows down the growth of bacteria. The term antibacterial is otherwise called as “antibiotic” [126]. It was coined by Selmon Waksman in 1942. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, a resistance to these drugs by microorganisms has increased. In general bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.

The problem of microbial resistance is growing and the outlook for the antimicrobial drugs in the feature is still uncertain. For a long period of time, plants have been a valuable source of natural products for maintaining human health. The use of both plant extract and phytoconstituents with known antimicrobial properties can be of great significance in therapeutic treatments. The various methods are available to screen the antibacterial activity. They are enumerate below

- Diffusion methods
  - Disc diffusion method
  - Hole plate diffusion method
- Dilution methods
  - Solid or agar dilution method
  - Liquid dilution method
- Bioautographic methods

These microbiological assays are based upon a comparison of the inhibition of growth of microorganism by measuring concentration of the antibiotics that are examined with that produced by known concentration of standard preparation of the antibiotic having the known activity.

The 70%ethanolic extract of *Ipomoea pes-tigridis* was screened for antibacterial activity [125] by disc diffusion method.

**Minimum Inhibitory Concentration (MIC) [127-129]**

**Bacteria**

The various organisms used in the present study include *Staphylococcus albus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa. Staphylococcus aureous,* and *Proteus spp*. These organisms were confirmed by biochemical tests.

**Preparation of media**

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/litre) Beef - 2 g, caesin acid hydrolysate 17.5g, starch 1.5g and agar 17 g; pH 7.4 ± 0.2.

MH agar (38g) was weighed and dissolved in 1000ml of distilled water and adjusted to pH 7.3 ± 0.2, sterilized by autoclaving at 121$^{o}$C for 15 minutes at 15psi pressure and was used for sensitivity tests.

**Preparation of bacterial cultures**

Few colonies of the bacterial strains selected for study were picked from the agar slopes and inoculated into 4ml peptone water in a test tube. These tubes were incubated for 2-4 hours to produce suspensions. The suspensions were then diluted, if necessary with saline to a density visually equivalent to that of standard prepared by adding 0.5ml of 1% barium chloride to 99.5ml of 1% sulphuric acid. These suspensions were used for seeding.

**Preparation of the extracts**

The plant extract was dissolved in DMSO to get a concentration of 10mg/mL.

**Preparation of agar plates**

The media (20mL) was introduced aseptically into sterilized petridishes [] and the petridishes were swirled until the agar begins to set.

**Disc Diffusion technique**

The pathogenic strains were then seeded on the MH agar media in a petridish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry.

**Test Procedure**

The plain sterile discs of 6mm diameter were obtained from Hi Media. The discs were then impregnated with different concentrations of the 70% ethanolic extract of *Ipomoea pes-tigridis* and solvent DMSO. Ciprofloxacin was used as a standard. Each disc contained 30μg. The standard, extract and DMSO discs were then placed on the seeded medium plates. The plates were then incubated at 37$^{o}$C for 24h. The results were read by the presence or absence of zone of inhibition. The zone of inhibition was then measured. The results for susceptibility tests, MIC and zones of inhibition are tabulated in **Tables 21 to 23** and **Fig. 26 to 32.**

## RESULTS AND DISCUSSION

### Anti-Oxidant Activity

### Method 1: DPPH assay method

The free radical scavenging power of 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid against DPPH was presented in **Table 14** and **Fig 18.**

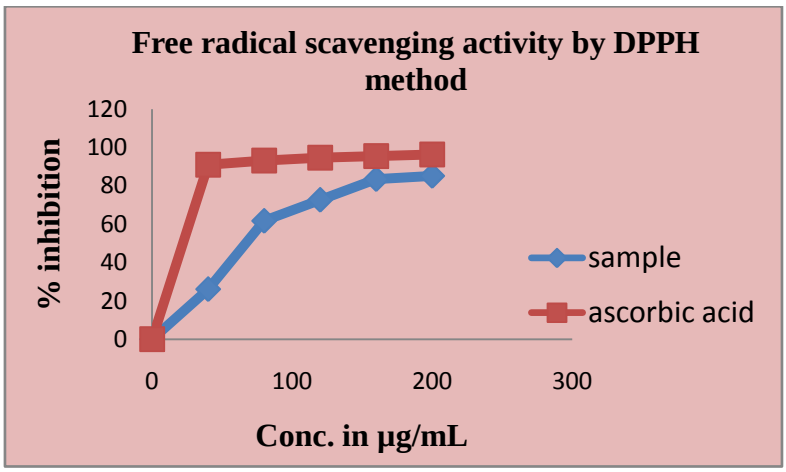
**Table 14: Free radical scavenging activity of 70% ethanolic extract of *I. pes-tigridis* by DPPH assay**

| S. No. | Conc. in µg/mL | Percentage inhibition by ascorbic acid | Percentage inhibition by test sample |
|---|---|---|---|
| 1 | 40 | 91.06±0.02 | 26.10±0.02 |
| 2 | 80 | 93.27±0.05 | 61.72±0.09 |
| 3 | 120 | 94.77±0.03 | 72.70±0.15 |
| 4 | 160 | 95.58±0.04 | 83.49±0.10 |
| 5 | 200 | 96.38±0.07 | 85.18±0.21 |
| | **$IC_{50}$** | **19.69µg/mL** | **88.82µg/mL** |

*** Mean of three readings ± SEM**

From the T**able 14**, it can be observed that a concentration of 200µg/mL produced percentage inhibition of 96.38±0.07 for ascorbic acid while it was 85.18±0.21% for the 70% ethanolic extract.

**Fig. 18: Free radical scavenging of 70% ethanolic extract of *I. pes-tigridis* against DPPH**



The $IC_{50}$ was calculated using the regression analysis was found to be 19.69µg/mL and 88.82µg/mL for ascorbic acid and extract respectively.

The DPPH contains one odd electron which becomes paired off in presence of free radical scavenger which reduces the characteristic absorption of DPPH at 517nm so decolourisation of the purple colour occurs. The free radical scavenging power of the extract increases as the concentration of the extract increases and showed more decolourisation and the solution turned yellow. Hence the free radical scavenging capacity of the extract is dose dependent.

**Method 2: Nitric oxide scavenging activity**

The free radical scavenging power of 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid against nitric oxide radical are presented in **Table 15** and **Fig. 19.**

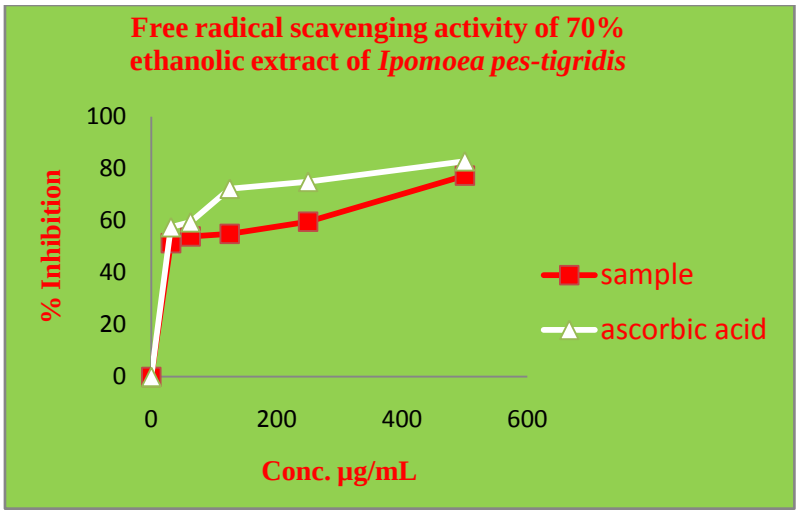
**Table 15: Percentage inhibition of 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid against nitric oxide**

| S. No. | Conc. in µg/mL | Percentage inhibition by ascorbic acid* | Percentage inhibition by test sample* |
|---|---|---|---|
| 1 | 31.25 | 57.58 ± 0.45 | 51.38 ± 0.32 |
| 2 | 62.5 | 59.47 ± 0.38 | 53.99 ± 0..50 |
| 3 | 125 | 72.38 ± 0.13 | 54.97 ± 0.21 |
| 4 | 250 | 75.04 ± 0 07 | 59.66±0.09 |
| 5 | 500 | 82.94 ± 0 .15 | 77.34 ± 0.12 |
| | **$IC_{50}$** | **86.85** | **165.96** |

***Mean of three readings ± SEM**

From **Table 15**, it can be observed that a concentration of 500µg/mL produced percentage inhibition of 82.94 ± 0.15 for ascorbic acid, while it was 77.34 ± 0.12 for the 70% ethanolic extract and the $IC_{50}$ calculated using the regression analysis was found to be 86.85µg/mL and 165.96µg/mL for ascorbic acid and 70% ethanolic extract respectively.

**Fig. 19: Nitric oxide radical scavenging by 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid**




In nitric oxide scavenging method, the formation of nitrite ion from nitro prusside which diazotizes the sulphanilamide and produce pink colour with a maximum absorption at 546nm. The percentage inhibition of free radical scavenging capacity of the extract will increase with increasing concentration of the extract. Hence the free radical scavenging capacity of the extract is a dose dependent

**Method 3: Reducing power assay (FRAP) method**

The results for the percentage increase reducing power of hydro alcoholic extract of *Ipomoea pes-tigridis* and ascorbic acid on potassium ferric cyanide are presented in **Table 16** and the pictorial representation of the same are presented in **Figs. 20 & 21.**

**Table 16: Reducing power of 70% ethanolic extract of *I. pes-tigridis* and ascorbic acid on potassium ferric Cyanide**

| S. No. | Conc. in µg/mL | % increase in reducing power of ascorbic acid | Conc. in µg/mL | % increase in reducing power of test sample |
|---|---|---|---|---|
| 1 | 10 | 20.87 ± 0.001 | 31.25 | 16.66 ± 0.01 |
| 2 | 15 | 34.46± 0.002 | 62.5 | 18.79 ± 0.02 |
| 3 | 20 | 53.40 ± 0.002 | 125 | 35.10 ± 0.05 |
| 4 | 25 | 85.92 ± 0.001 | 250 | 54.25 ± 0.07 |
| 5 | 30 | 99.03 ± 0.001 | 500 | 88.62 ± 0.01 |
| | **IC 50** | **16.97 µg/mL** | **IC 50** | **247.89 µg/mL** |

*** Mean of three readings ± SEM**

From **Table 16,** it can be observed that a concentration of 30µg/mL produced a percentage inhibition of 99.03 ± 0.001 for ascorbic acid while it was 88.62 ± 0.01 at a concentration of 500µg/mL for the 70% ethanolic extract and the $IC_{50}$ was calculated using the regression analysis. It was found to be 16.97µg/mL and 247.89µg/mL for ascorbic acid and 70% ethanolic extract respectively.

**Fig.20: Reducing power assay of ascorbic acid on potassium ferriccyanide**

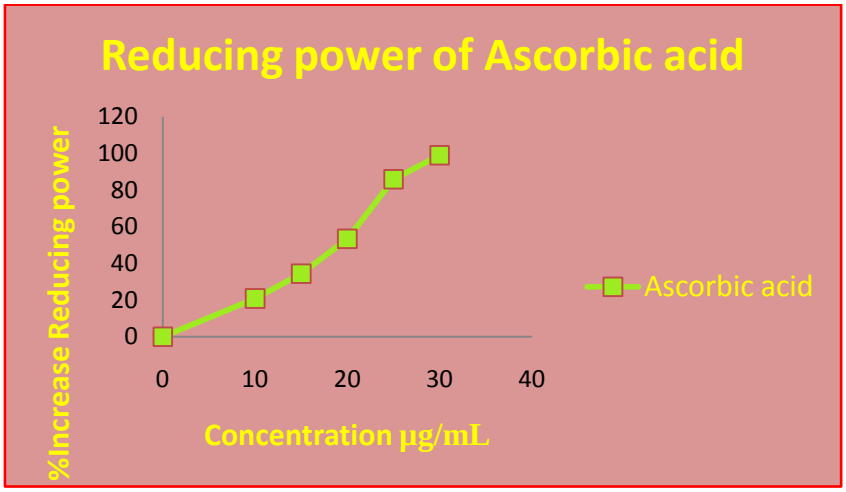
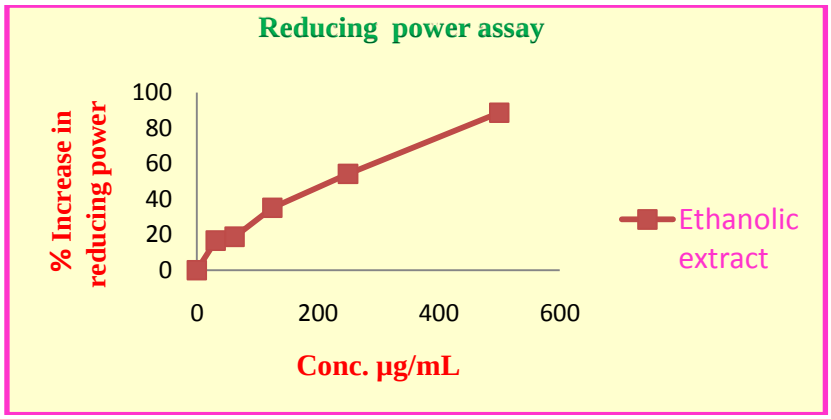



**Fig.21: Reducing power assay of 70% ethanolic extract of *Ipomoea pes-tigridis* on potassium ferricyanide**




In reducing power assay method (FRAP), the reduction of ferric in potassium ferricyanide to ferrous to form potassium ferrocyanide by the sample and the subsequent

formation of Prussian blue colour with ferric chloride increases with concentration increases. So the reducing power of the extract is a dose dependent.

**Method 4: Free radical scavenging activity against hydrogen peroxide**

The free radical scavenging power of 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid against hydrogen peroxide are presented in **Table 17** and **Fig. 22.**

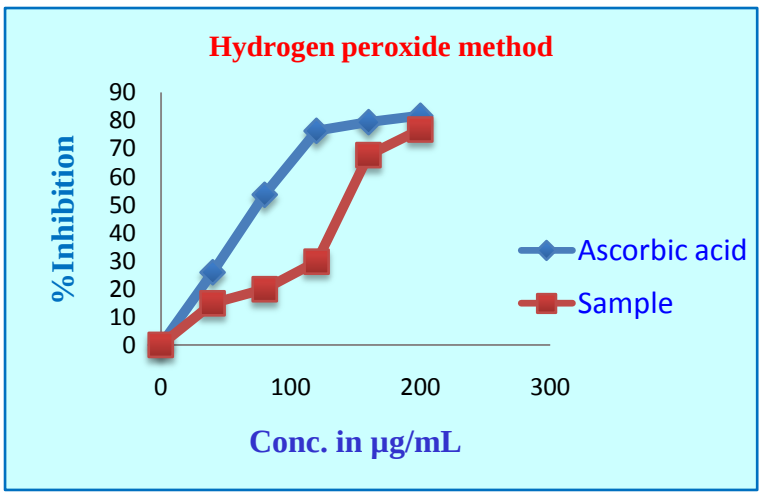
**Table 17: Percentage inhibition of 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid against hydrogen peroxide**

| S. No. | Conc. in µg/mL | Percentage inhibition | |
|---|---|---|---|
| | | Test sample | Ascorbic acid |
| 1 | 40 | 14.84 ± 0.06 | 18.81±0.002 |
| 2 | 80 | 19.84 ± 0.09 | 23.52±0.001 |
| 3 | 120 | 29.72 ± 0.12 | 47.85±0.002 |
| 4 | 160 | 67.69 ± 0.32 | 72.18±0.001 |
| 5 | 200 | 76.80 ± 0.18 | 86.56±0.003 |
| | **$IC_{50}$** | **138.47µg/mL** | **93.32 µg/mL** |

***Mean of three readings ± SEM**

From **Table 17**, it can be observed that a concentration of 200µg/mL produced percentage inhibition of 86.56±0.003 for ascorbic acid while it was 76.80 ± 0.18 for the 70% ethanolic extract and the $IC_{50}$ was calculated using the regression analysis and was found to be 93.32µg/mL and 138.47µg/mL for ascorbic acid and 70% ethanolic extract respectively.

**Fig. 22: Percentage inhibition of hydrogen peroxide by 70% ethanolic extract of *Ipomoea pes-tigridis* and ascorbic acid**



The capacity of the extract to decompose the hydrogen peroxide to water increases with increasing concentration of the extract. Hence the scavenging power of the extract is a dose dependent. The plant extract possesses good antioxidant property which may be attributed to the presence of phenolic compounds and flavanoids which have been estimated quantitatively.

All the above methods the antioxidant property of the extract is comparable as that of standard ascorbicacid. Hence the 70% ethanolic extract of *Ipomoea pes-tigridis* showed good antioxidant activity.

**Anti-Inflammatory Activity**

The results for the percentage inhibition of membrane stabilization of extract and diclofenac sodium were depicted in **Table 18** and **Fig. 23.**

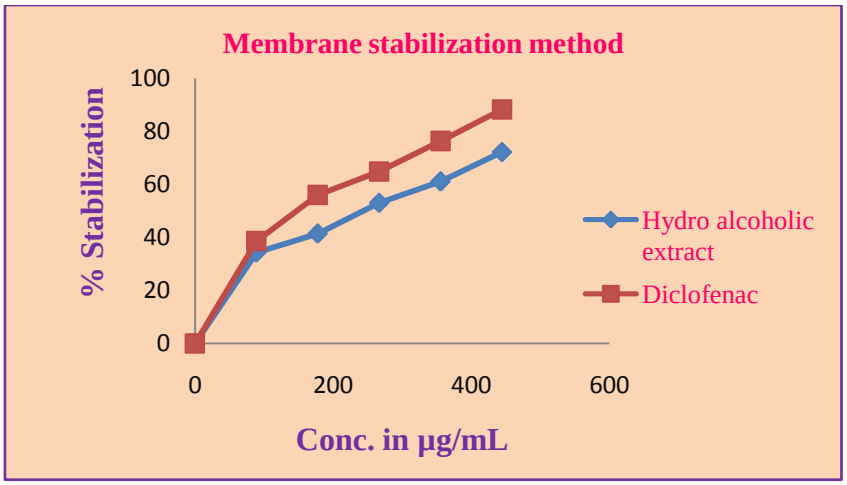
**Table 18: Percentage of membrane stabilization diclofenac and 70% ethanolic extract of *Ipomoea pes-tigridis***

| S. No. | Conc. in µg/mL | Percentage membrane stabilization of diclofenac | Percentage membrane stabilization of extract |
|---|---|---|---|
| 2 | 88.88 | 34.25± 0.07 | 38.65± 0.03 |
| 2 | 177.77 | 41.42 ± 0.16 | 56.02 ±0.12 |
| 3 | 266.66 | 52.98 ± 0.04 | 64.84 ±0.21 |
| 4 | 355.55 | 61.09 ± 0.13 | 76.34 ±0.26 |
| 5 | 444.44 | 72.14 ± 0.27 | 88.23 ±0.16 |

***Mean of three readings ± SEM**

From **Table 18,** it can be observed that at a concentration of 444.44µg/mL, the percentage membrane stabilization was 76.14 ± 0.27 for the extract and 88.23 ±0.16 for diclofenac sodium.

**Fig. 23: Percentage of membrane stabilization by diclofenac and 70% ethanolic extract of *Ipomoea pes-tigridis***




In many inflammatory disorders, there is excess production of superoxide radical, hydroxide radicals as well as hydrogen peroxide [101] which harm tissues severely either by direct oxidising action or indirectly through the production of $H_2O_2$ & OH radical from $O^{2-}$ which initiates lipid peroxidation which results in membrane destruction. This then provokes the inflammatory process by production of mediators and chemotactic factors [102]. The ROS also activate metallo proteinase damage which has been observed in arthritic tissues

The mechanism of anti- inflammatory agents is the inhibition of COX enzyme which is responsible for conversion of arachidonic acid to prostaglandins. The NSAIDs act by either inhibiting the lysosomal enzymes (COX) or by stabilising the membrane. The extract exhibited the membrane stabilisation effect by inhibiting the hyposaline and heat induced lysis of erythrocyte membrane which is considered analogues to lysosomal membrane.

Stabilisation of lysosomal membrane is important which limits the inflammatory response by preventing the release of the constituents present in the lysosome namely bactericidal enzymes & proteases which further inflame and damage the tissue on extracellular release. Some of the NSAIDs are known to exhibit membrane stabilisation due to osmotic loss of intracellular electrolytes and fluid components [103, 104].

The results obtained in the above study indicate that *Ipomoea pes-tigridis* can be a good source of anti-inflammatory drug relatively safe for consumption and also as a source of natural anti oxidant agent.

**Anti Arthiritic activity**

***In vitro* Protein Denaturation Method**

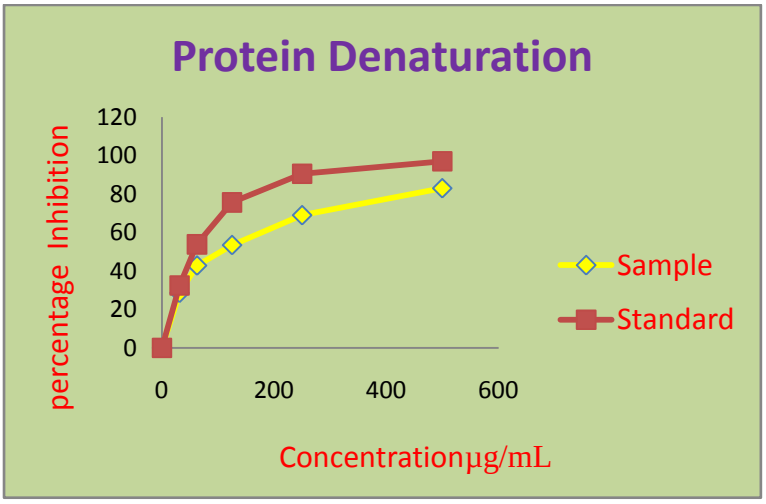
The results of anti arthritic activity by inhibition of protein denaturation method are presented in **Table 19** and the graphical representation is depicted in **Fig. 24.**

**Table 19: Effect of 70% ethanolic extract of *I. pes-tigridis* and diclofenac on inhibition of protein denaturation**

| S. No | % inhibition | | |
|---|---|---|---|
| | Conc. in µg/ml | 70% ethanolic extract | Diclofenac |
| 1 | 31.25 | 28.56± 2.35 | 32.48 ± 2.44 |
| 2 | 62.5 | 42.78± 2.16 | 53.85 ± 1.11 |
| 3 | 125 | 53.45± 1.45 | 75.64 ± 1.21 |
| 4 | 250 | 68.96 ±1.89 | 90.60 ± 2.12 |
| 5 | 500 | 82.98 ± 3.32 | 97.01 ± 1.39 |
| | **IC50** | **189.63µg/mL** | **110.32µg/mL** |

***Mean of three readings ± SEM**

**Fig. 24: Percentage inhibition of protein denaturation by Diclofenac And 70% ethanolic extract of *Ipomoea pes-tigridis***



From the above results, it can be seen that the percentage inhibition of protein denaturation by 70% ethanolic extract of *Ipomoea pes-tigridis* was 82.98 ± 3.32 while for diclofenac, it was 97.01 ± 1.39 at IC. As the ethanolic extract possessed both *in vitro* membrane stabilisation and *in vitro* protein denaturation effect, it will be beneficial for rheumatoid arthritis activity.

Protein denaturation is a process in which the proteins lose their $2^{o}$& $3^{o}$ structure by application of external stress (such as reacting with an acid or base, heat etc). Most biological proteins lose their function when denatured.

Proteinases have been implicated in arthritic reactions neutrophile are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It has been also reported that leucocyte proteinases play arole in the development of tissue damage during the inflammation process and significant protection was provided by proteinase inhibitors [112].

Denaturation of proteins is also to be a reason for inflammation. The inflammatory drugs such as phenyl butazone and salicylic acid have shown to possess the ability of inducing protein denaturation thermally [113]

The results obtained in the above study indicate that *Ipomoea pes-tigridis* can be a good source for the treatment of arthritics .

**Cytotoxic Activity**

***In vitro* anti cancer activity (liver cancer) by MTT assay**

The cytotoxic activity of 70% ethanolic extract of *Ipomoea pes-tigridis* on liver cancer cell lines by MTT assay which was presented in **Table 20** and **Figs. 25 & 26.**

**Table 20: Cytotoxic activity using liver HEP G2 cell lines by MTT assay method**

| S. No | Conc. µg/mL | % cell inhibition | $IC_{50}$ µg/mL |
|---|---|---|---|
| 1 | 31.25 | 0.3646 | **155.2** |
| 2 | 62.5 | 3.2818 | |
| 3 | 125 | 35.4604 | |
| 4 | 250 | 79.7630 | |
| 5 | 500 | 98.3592 | |

**Fig. 25: Cytotoxic activity (% growth inhibition) using Liver HEP G2 cell lines by MTT assay method**

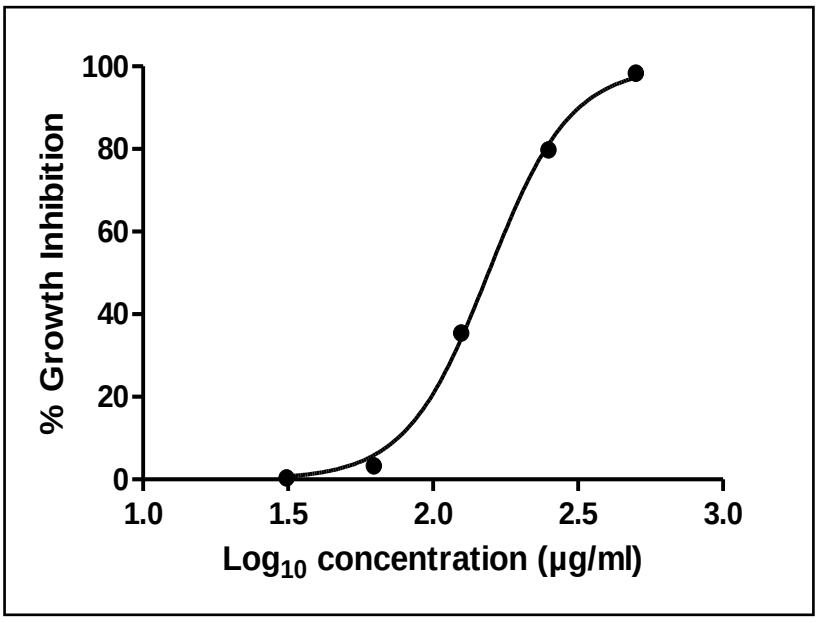
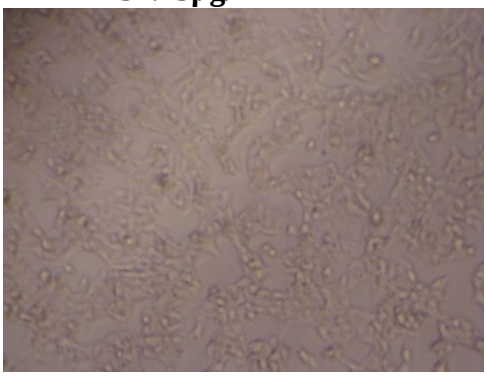
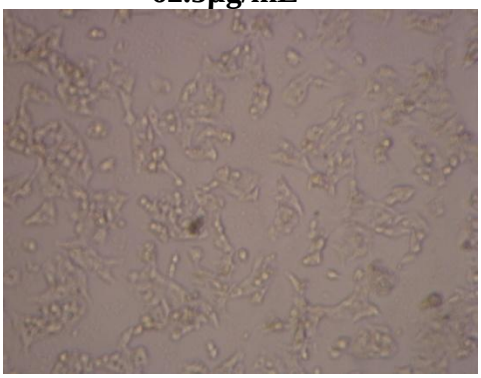


**Fig. 26: Cell inhibition (formazan crystals produced) at various concentrations of 70%ethanolic extract after MTT treatment by using HEPG2 cell lines**

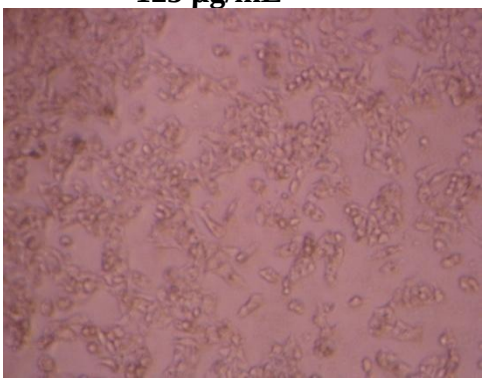
**31.25µg/mL**



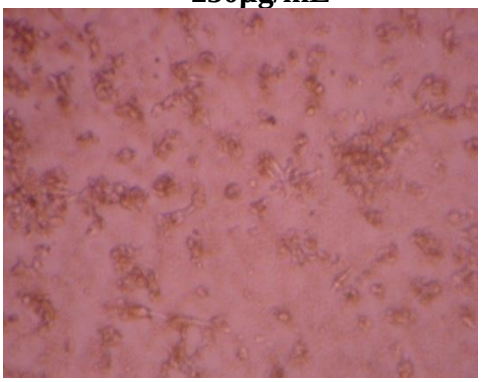
**62.5µg/mL**



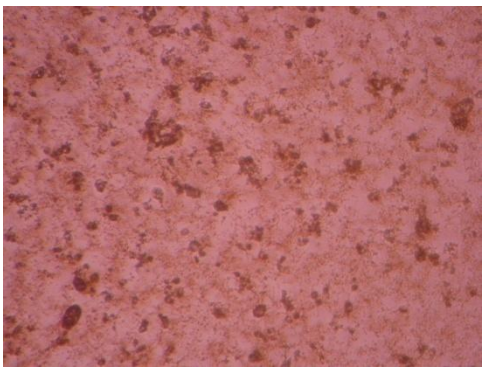
**125 µg/mL**



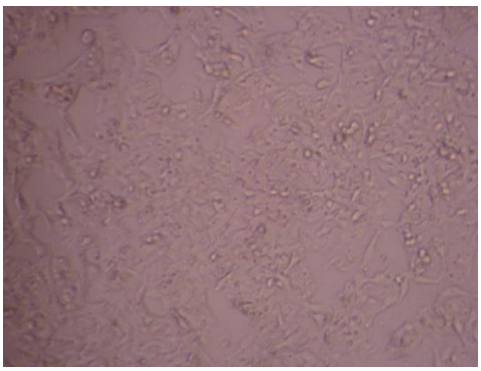
**250µg/mL**



**500 µg/mL**



**CONTROL**



The amount of formazan crystals produced by MTT is directly proportional to the number of viable cells which and the same is depicted in **Fig. 26.** A decrease in the cell count was observed with the increase in the concentration of the extract. There was a dose depended increase in the cytoxic activity. The extract at low concentration (31.25 µg/mL) showed 0.36 % cell inhibition and at high concentration (500 µg/mL) 98.83% cell inhibition

and the same is seen in **Table 20.** The inhibitory concentration ($IC_{50}$) value was calculated using the regression analysis and was found to be155.2µg/mL which is shown in **Fig. 25.**

The 70% ethanolic extract of *Ipomoea pes-tigridis* was studied for their *in vitro* effects on liver HEPG2 cell lines. The selection of the crude plant extracts for screening has the potential of being more successful in initial steps than the screening of pure compounds which are isolated from natural products [120, 121]. In this study the 70% ethanolic extract exhibited the most effective cytotoxicity at 500µg/mL (98.83%) which is depicted in **Table 20.** From the above study the crude extract has pharmacological effect [122] which may be due to the synergistic effects of the various components present in the crude extract. The active constituents namely flavanoids and terpenoids may be responsible for reducing the cancer risk factors [123-125]. Thus the 70% ethanolic extract of *Ipomoea pes-tigridis* exhibited significant anti cancer effect particularly for liver cancer.

**Antibacterial activity**

The results obtained for the susceptibility tests of the extract against various microorganisms are presented in **Tables 21 to 23** and photographic documentation pertaining to this are presented in **Fig. 27 to 32.** From the **Table 21,** it can be seen that there was no growth against all the tested micro organisms at a concentration of1.5mg/disc.

**Table 21: Susceptibility tests of 70%ethanolic extract of *Ipomoea pes-tigridis* for various Microorganisms**

| S. No | Name of the treatment | Conc. in mg/disc | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|
| 1 | Control | ---- | + | + | + | + | + | + |
| 2 | Ciprofloxacin | ---- | + | + | + | + | + | + |
| 3 | 70% Ethanolic extract of *I. pes-tigridis* | 0.5 | + | + | + | + | + | + |
| | | 1.0 | + | + | + | + | + | + |
| | | 1.5 | - | - | - | - | - | - |

**NOTE:**- (+) indicates growth; (-) indicates no growth

1. *Escherichia coli* 2. *Klebsiella pneumoni 3. Proteus mirabilis 4. Staphylococcus aureus 5. Staphylococcus albus* 6. *Pseudomonas aeurginosa*

**Table 22: MIC of 70%ethanolic extract of *I. pes-tigridis* against various microorganisms**

| S. No | Name of the organism | Minimum inhibitory concentration (in mg/disc) |
|---|---|---|
| 1. | *Escherichia coli* | 1.5 |
| 2. | *Klebsiella pneumoniae* | 1.5 |
| 3. | *Proteus mirabilis* | 1.5 |
| 4. | *Staphylococcus aureous* | 1.5 |
| 5 | *Staphylococcus albus* | 1.5 |
| 6 | *Pseudomonas aeruginosa* | 1.5 |

From the **Table 22,** it can be observed that the MIC for all the six tested organisms *E. coli, Klebsiella pneumonia, proteus mirabilis, Staphylococcus aureus, Staphylococcus albus, Pseudomonas aeurginosa* were found to be 1.5mg/disc.

**Table 23: Antibiotic disc diffusion assay against various microorganisms**

| S. No | Name of the organism | Zone of inhibition (in mm)* | |
|---|---|---|---|
| | | Standard | 70%ethanolic extract |
| 1. | *Escherichia coli* | 26.0 ± 0.0 | 24.0 ± 0.5 |
| 2. | *Klebsiella pneumonia* | 26.0 ± 1.0 | 24.0 ± 0.5 |
| 3. | *Proteus mirabilis* | 25.0 ± 0.0 | 19.0± 0.5 |
| 4. | *Staphylococcus aureus* | 26.0 ± 0.5 | 18.0 ± 0.5 |
| 5. | *Staphylococcus albus* | 26.0 ± 0.0 | 24.0 ± 0.5 |
| 6. | *Pseudomonas aeruginosa* | 28.0 ± 0.0 | 22.0 ± 0.5 |

*** mean of 2 readings ± SEM**

The results obtained for the antibiotic disc diffusion technique are presented in **Table 23** and **Figs. 27 to 32.** From the **Table 22**, it can be observed that the zones of inhibition of

the 70% ethanolic extract of *Ipomoea pes-tigridis* for the tested organisms were comparable to that of standard Ciprofloxacin.

From the above study, the 70% ethanolic leaf extract of *Ipomoea pes-tigridis* inhibited the growth of above tested organisms at a concentration of 1.5mg/mL and also the extract has showed more potent activity against *E. coli, Klebsiella pneumonia and Staphylococcus albus* and moderate activity against *Proteu mirabilis, Staphylococcus aureus* and *Pseudomonas aeurginosa.*

**Secondary metabolities responsible for pharmacological activity**

The flavonoids isolated from different plants possess a wide spectrum of activity. They have been found to possess anti-oxidant, anti-microbial, hypolipedimic [145,146] anti cancer, aptosis inducing effects [150,151] and anti-inflammatory activities according to various research articles [130- 132]. The presence of terpenoids in plants has shown to possess antimicrobial and anti- inflammatory properties [133, 134]. Tannins in plants have also been suggested to possess potent antimicrobial activities [135] Anti- oxidant [136] and anti-inflammatory properties [137] and wound healing property [149]. Several alkaloids had been reported to possess anti diabetic effect [138, 139]. Saponins have also been indicated to posses potent anti-microbial activity [140, 141] antioxidant activity [142] anti-inflammatory activity [143] hypolipedemic activity [145,146] and haemolytic activity, cholesterol binding properties and bitterness [147, 148]. Studies showed that many flavanoids and poly phenolic compounds have shown to possess antioxidant and anti inflammatory activity [144]. Studies have revealed that triterpenoids have hypolipedemic activity [145,146]

In the present study, the preliminary phytochemical evaluation of powder and 70% ethanolic extract of *Ipomoea pes-tigridis* revealed the presence of steroids, phenolic compounds, flavonoids, terpenoids, tannins, carbohydrates proteins etc. A quantitative estimation of the total phenolic compounds and flavanoids showed that the extract possessed

an amount of 51.41± 5.37mg GAE/g and 23.59± 0.066 mg of quercetin equivalent/g of extract respectively.

As many authors have attributed that flavonoids, phenolic compounds, tannins and sterols to possess antioxidant, anti- inflammatory, anti arthritic, antibacterial and anticancer properties, the presence of the same in the 70% ethanolic extract of *Ipomoea pes-tigridis* may also be responsible for the activity.

# CHAPTER - 8

## SUMMARY AND CONCLUSION

This dissertation entitled "**Pharmacognostic, Phytochemical and Pharmacological evaluation on the leaves of *Ipomoea pes-tigridis* Linn. (Convolvulaceae).**

The chapter on **Review of literature** deals with information from the primary and secondary sources of investigation on the pharmacognostic and pharmacological work ***Ipomoea pes-tigridis*** and other related species.

The chapter on **Pharmacognostic Evaluation** deals with macroscopic and microscopic features, quantitative microscopy, and analytical parameters etc. The following features have been observed for *Ipomoea pes-tigridis*.

- The leaves are hairy, palmately 5-9 lobed; flowers are while and funnel shaped
- The leaf consists of a thick, abaxially hanging midrib, thick squarish epidermal cells, and parenchymatous ground tissue.
- The vascular strand is single bowl shaped and bicollateral and surrounded by hyaline bundles of sheath cells.
- Calcium oxalate crystals of druses or sphaero crystals are located in the spongy parenchyma.
- The epidermal cells appear amoeboid in outline and are wide with thick, wavy anticlinal walls.
- The stomata are predominantly paracytic type.
- The lateral veins are thin and straight and form reticulate venation. The vein-islets are rectangular or polygonal. The vein terminations are curved and are undulate

- The distal part of the petiole is semi circular along the abaxial side and has median shallow groove on the adaxial side and consists of parenchymatous, vascular strand having xylem and phloem elements.
- The evaluation of powder under microscopy showed the presence of unicellular and unbranched non-glandular epidermal trichomes, subsessile glandular trichomes, peltate-type glands are seen detached from the epidermis.

The observed macroscopical, microscopical, cytomorphological features have added more weightage to the authentication of the plant. Quantitative analytical microscopy, powder analysis and standardization parameter evaluation gives values which are constant and will be useful for the identification for its quality and purity, authentication of the crude drug and the plant material.

The chapter on **Phytochemical Evaluation** deals with the screening of the leaf powder and various extracts for the presence of phytochemical constituents. The quantitative estimation of phytoconstituents throws light on the type of secondary metabolites present in the plant and the quantification helps in determining the amount of certain type of secondary metabolites (phenolic compounds and flavanoids) which may be responsible for the pharmacological activity. **TLC** and **HPTLC** fingerprint profiles are useful for the impurity profiling of the crude drug in future.

The chapter on **Pharmacological Evaluation** deals with the screening of the 70% ethanolic extract of ***Ipomoea pes-tigridis*** for *in vitro* antioxidant, anti inflammatory, anti arthritic, anti proliferative and anti bacterial activities.

The extract possessed good **antioxidant** properties which may be due to the presence of phenolic and flavonoids in the extract. A very good *in vitro* membrane stabilization effect and protein denaturation effect showed that the plant extract possessed **anti inflammatory**

and **antiarthritic** properties which has been seen with other species of plants of the genus I*pomoea*.

The **anti cancer effect** particularly against **liver cancer on HEP G2** cell lines showed that the plant possesses anti proliferative effect comparable to that of *Ipomoea stans*.

The **antibacterial activity** has shown that the extract has a broad spectrum of activity against the tested gram positive and gram negative organisms. The pharmacological activities of the plant extract may be attributed to the free radical scavenging activity and due to presence of secondary metabolites like phenolic and flovanoids.

From the present work, it may be concluded that wide spectrum of activity of the plant extract has thrown light on the importance of the little known herb when compared to other species of the genus *Ipomoea*. The future scope of the work is to isolate the flavonoid responsible for the activity and also to carry out the pharmacological screening *in vivo* models.

**FIG . 1. HERBARIUM OF *Ipomoea pes-tigridis* Linn**

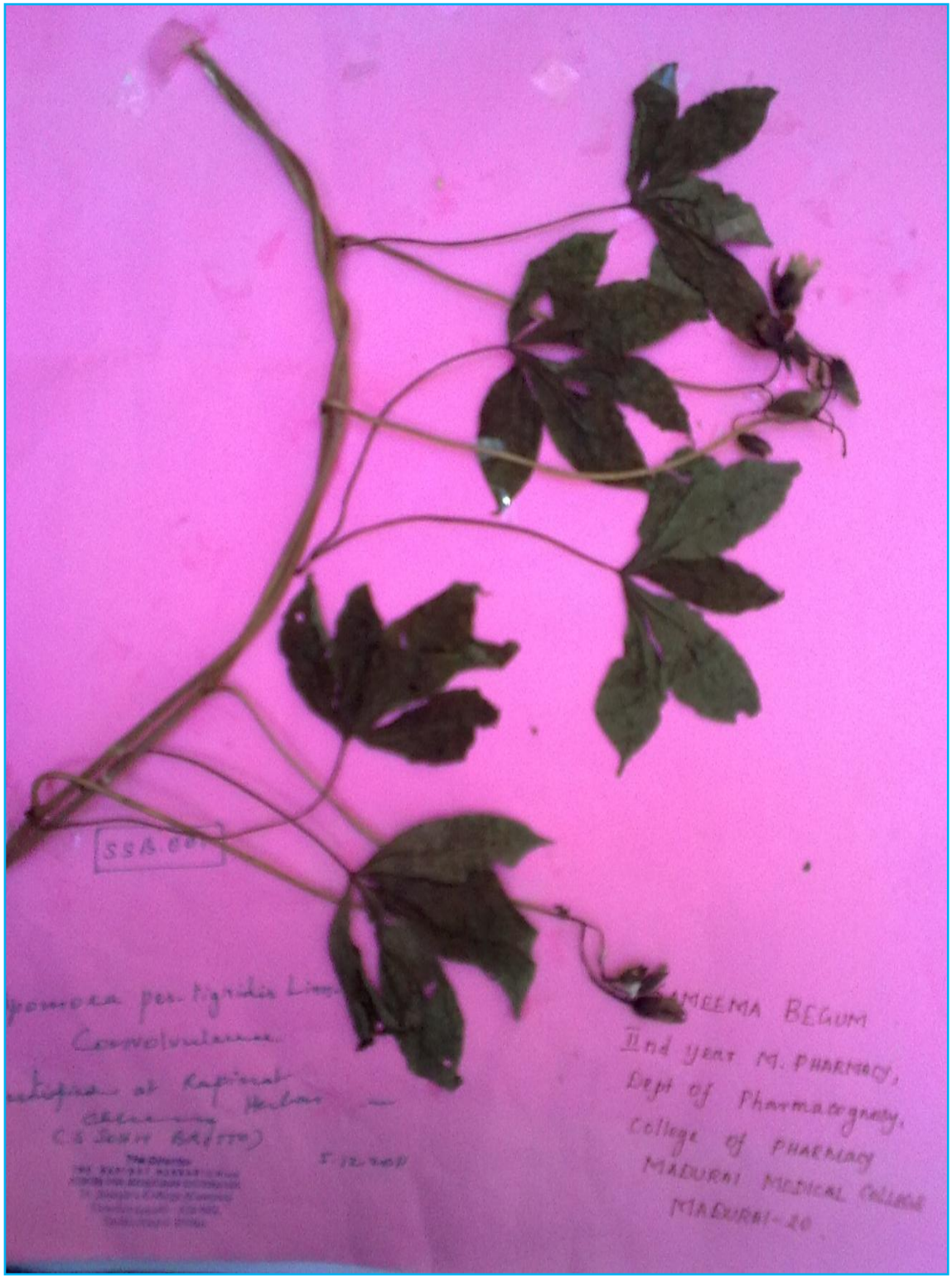


**FIG. 2.1: HABITAT OF *IPOMOEA PES-TIGRIDIS***

**FIG. 2.2: HABITAT OF IPOMOEA PES-TIGRIDIS**

FIG. 2.3: ROOT

FIG. 2.4: FLOWER

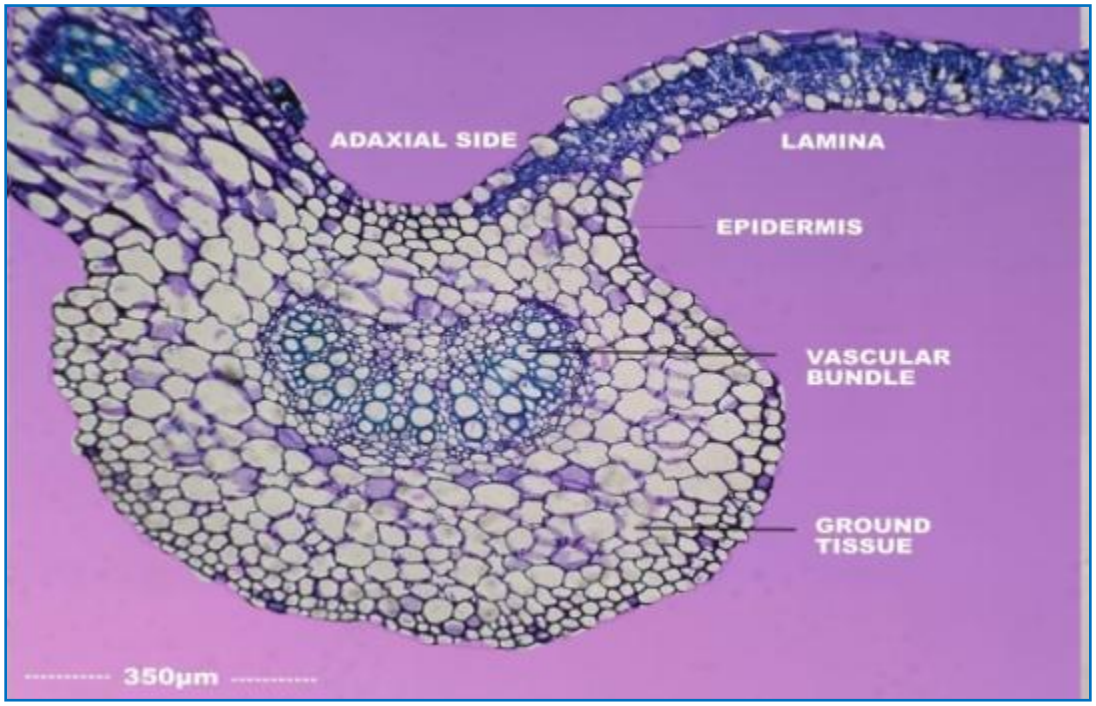
FIG. 2.5: UNRIPE FRUIT

FIG. 2.6: FRUIT

**FIG. 2.7: LEAF - DORSAL VIEW**

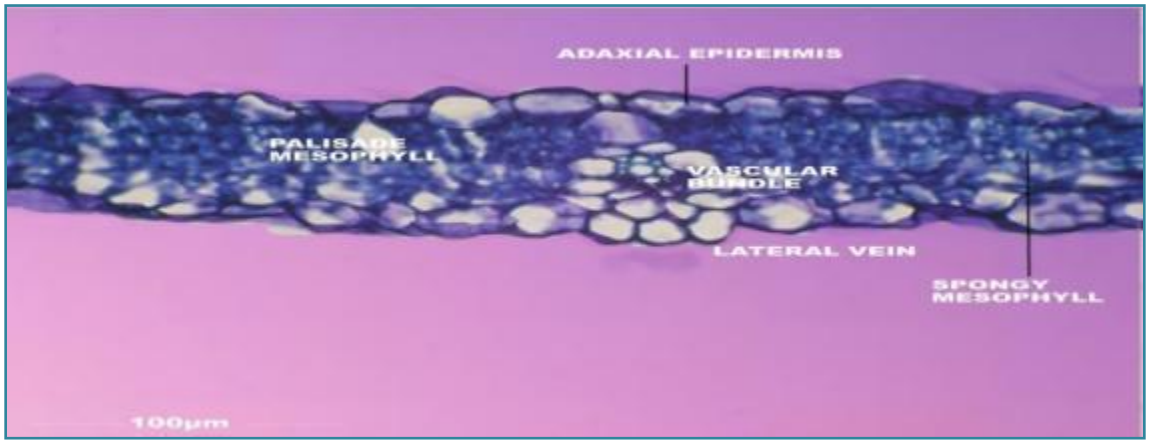
**FIG. 2.8: LEAF- VENTRAL VIEW**

## FIG. 3.1: TS OF LEAF THROUGH MIDRIB

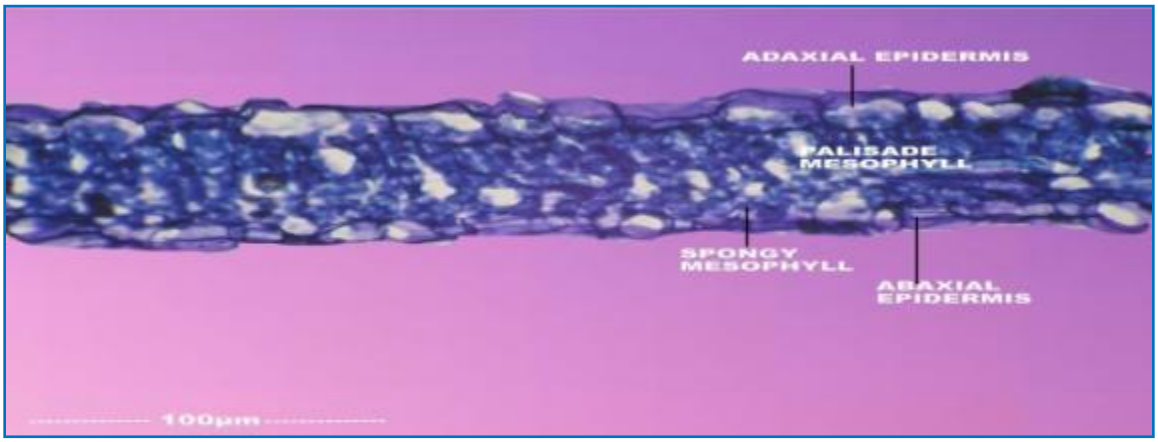



## FIG. 3.2: VASCULARBUNDLE OF THE MIDRIB ENLARGED

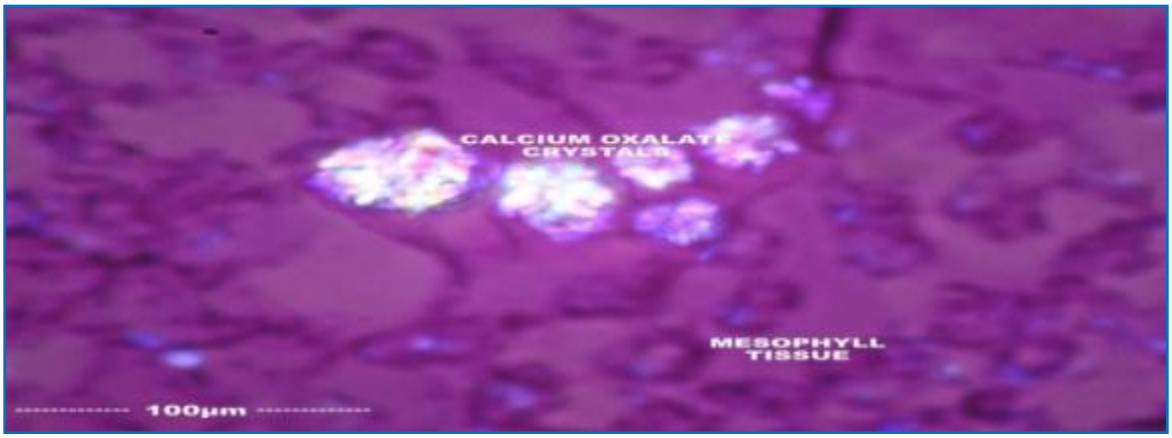
## FIG. 4.1: TS OF LEAF THROUGH LATERAL VIEN



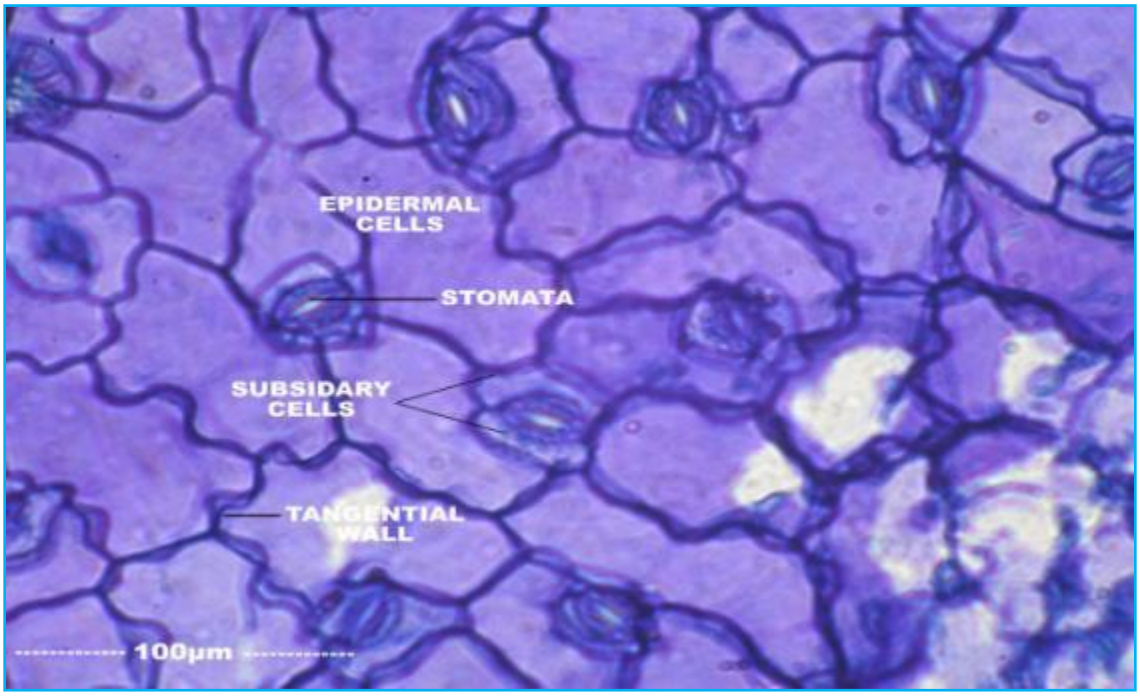

## FIG. 4.2: TS OF LAMINA



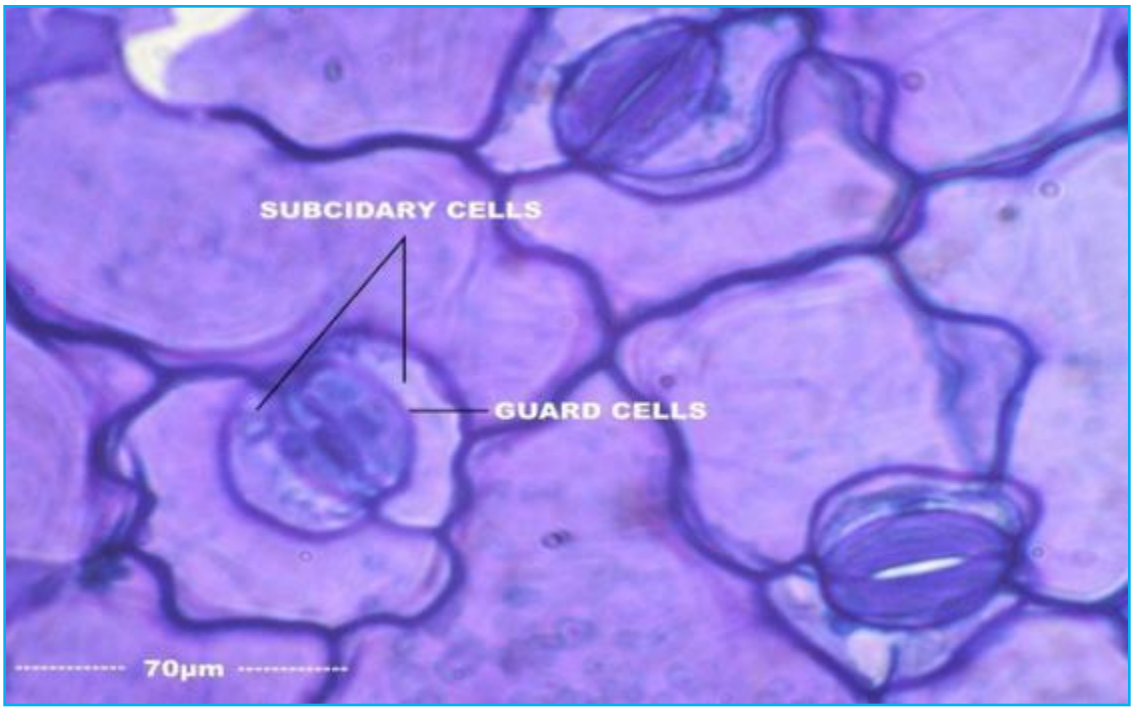

## FIG. 4.3: CALCIUM OXALATE DRUSES WITH LEAF MESOPHYLL



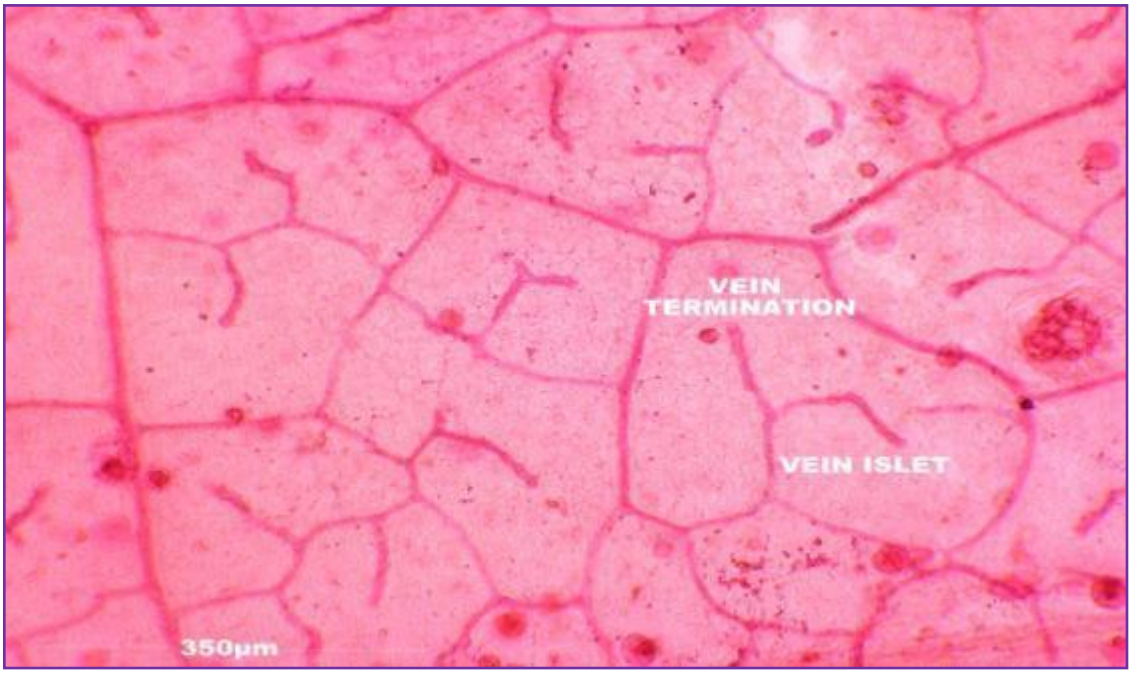
## FIG.5.1: EPIDERMIS PARADERMAL SECTION



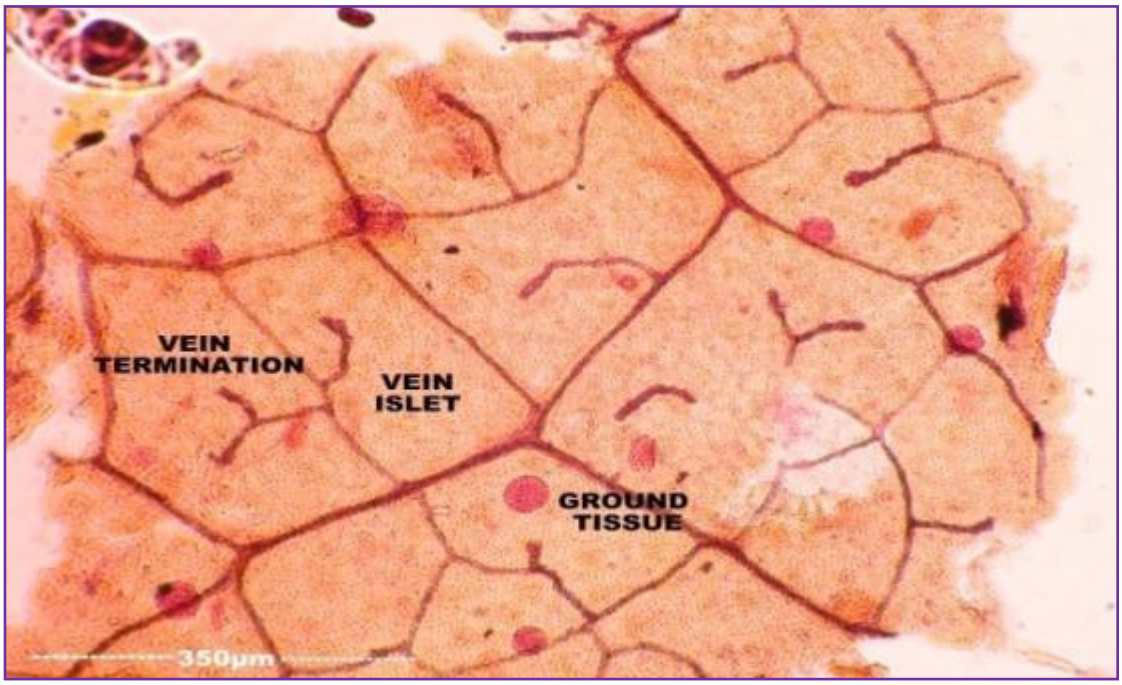

## FIG. 5.2: STOMATA ENLARGED



## FIG.6.1: VENATION PATTERN

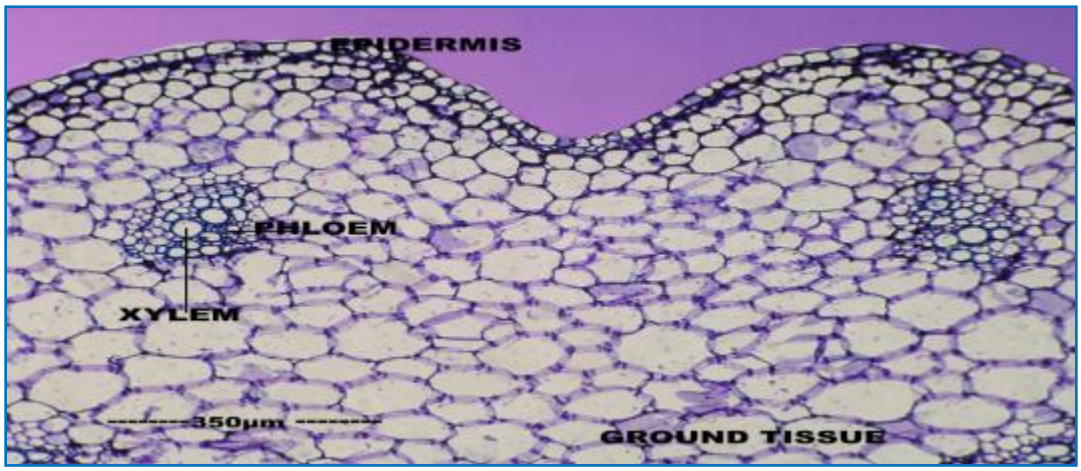



## FIG 6.2: VENATION PATTERN

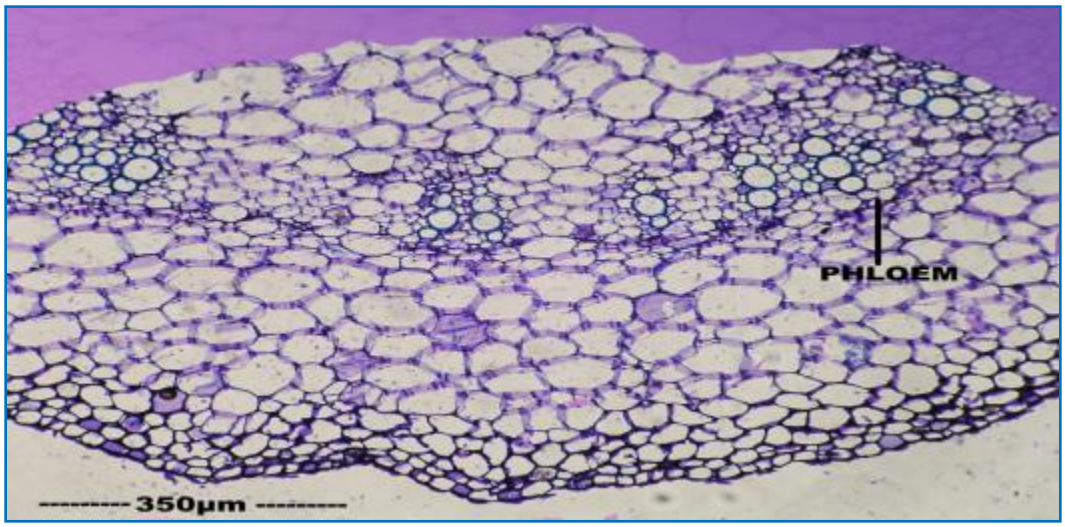


## FIG. 7.1: T.S OF DISTAL PART OF THE PETIOLE


## FIG. 7.2: UPPER PART OF THE PETIOLE




## FIG. 7.3: LOWER PART OF THE PETIOLE



## FIG.8: T.S OF PROXIMAL PART OF THE PETIOLE

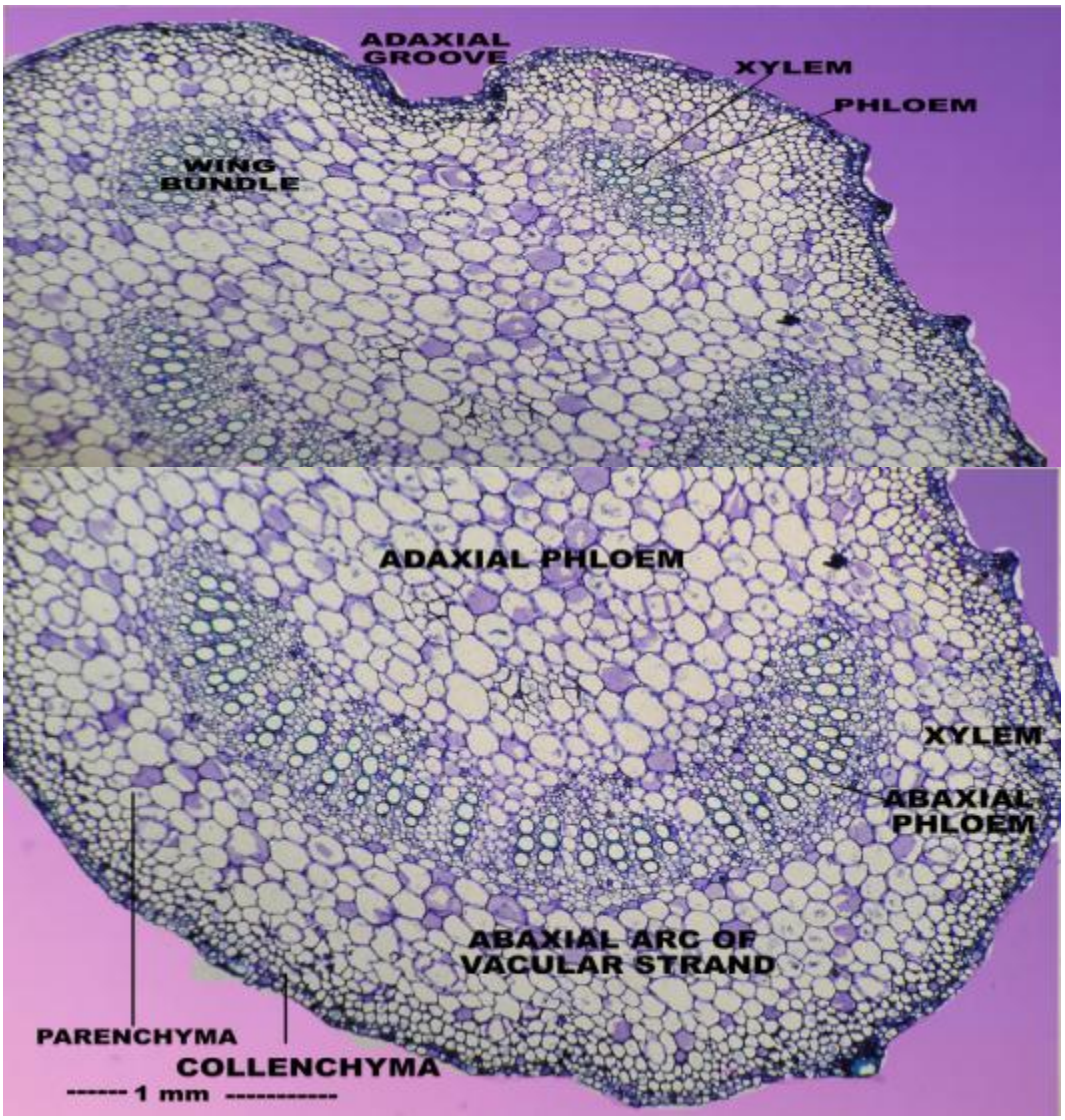


**FIG. 9.1: A PORTION OF THE PETIOLE ENLARGED**

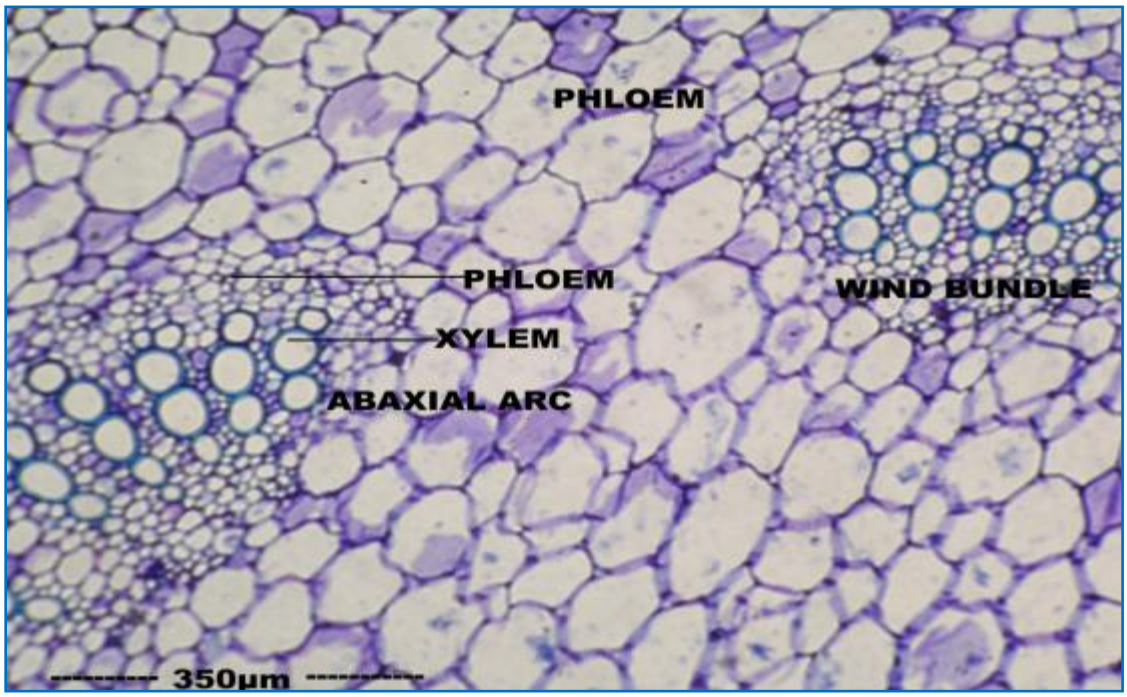
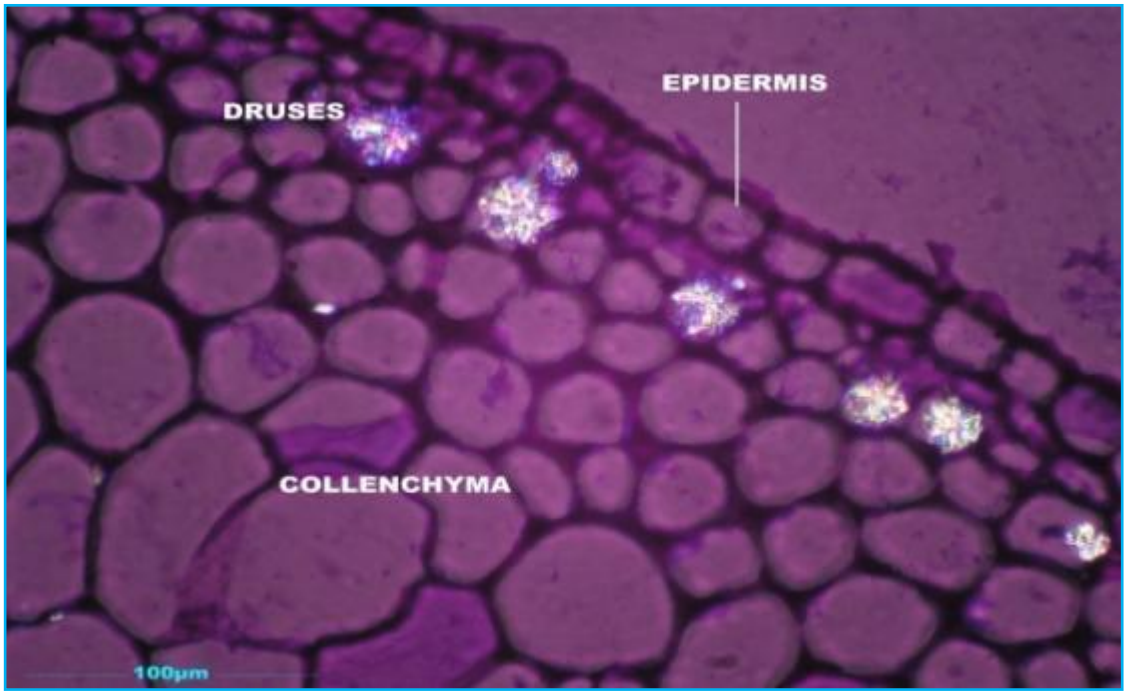
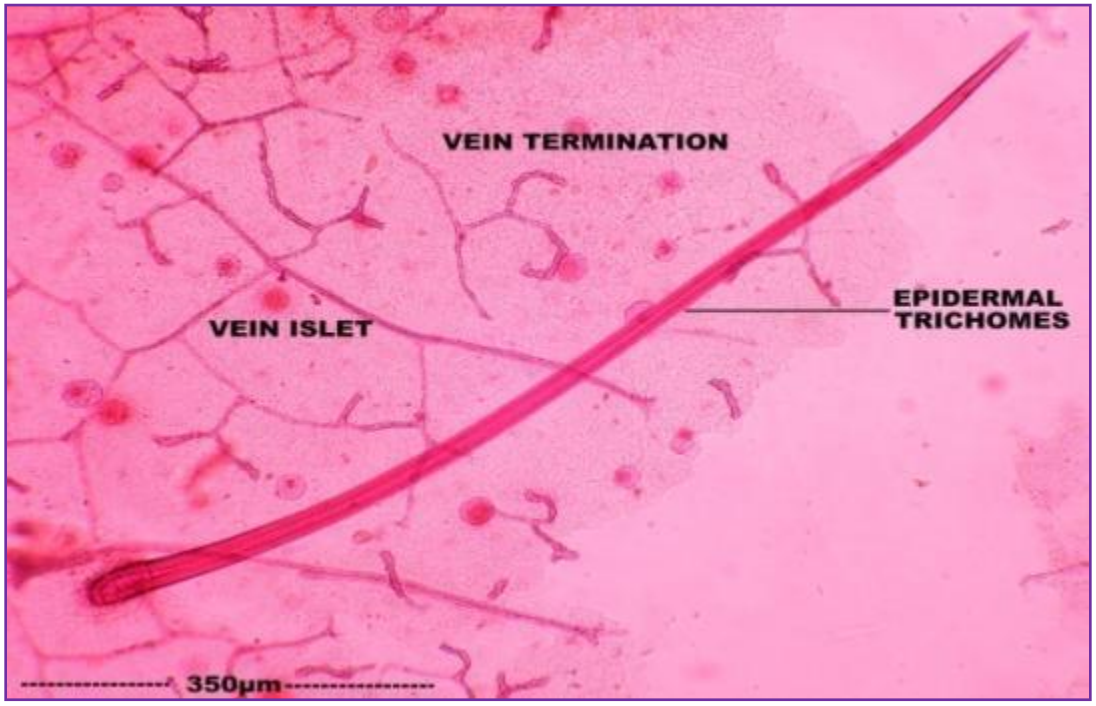


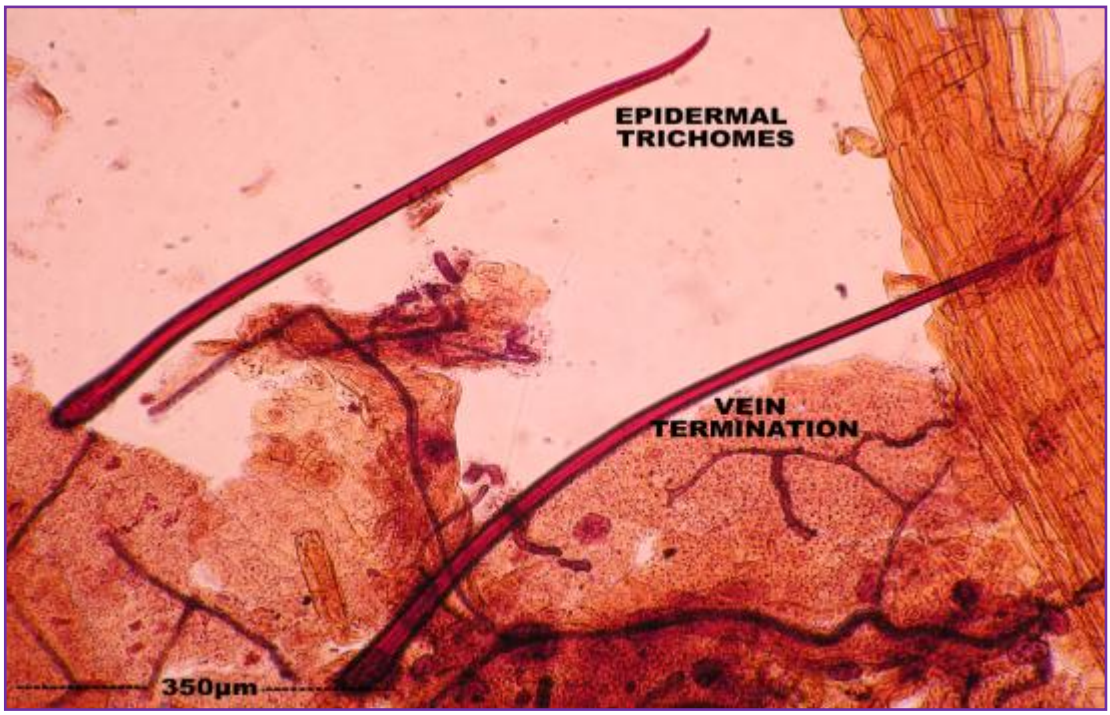

**FIG. 9.2: CALCIUM OXALATE CRYSTALS IN THE EPIDERMAL PORTION OF THE LAMINA**



## FIG.10.1: VENATION OF THE LAMINA AND EPIDERMAL TRICHOME




## FIG. 10.2: EPIDERMAL TRICHOMES



## FIG. 11.1: GLANDULAR TRICHOMES ON THE LAMINA


## FIG. 11.2: GLANDULAR TRICHOMES ON THE LAMINA

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